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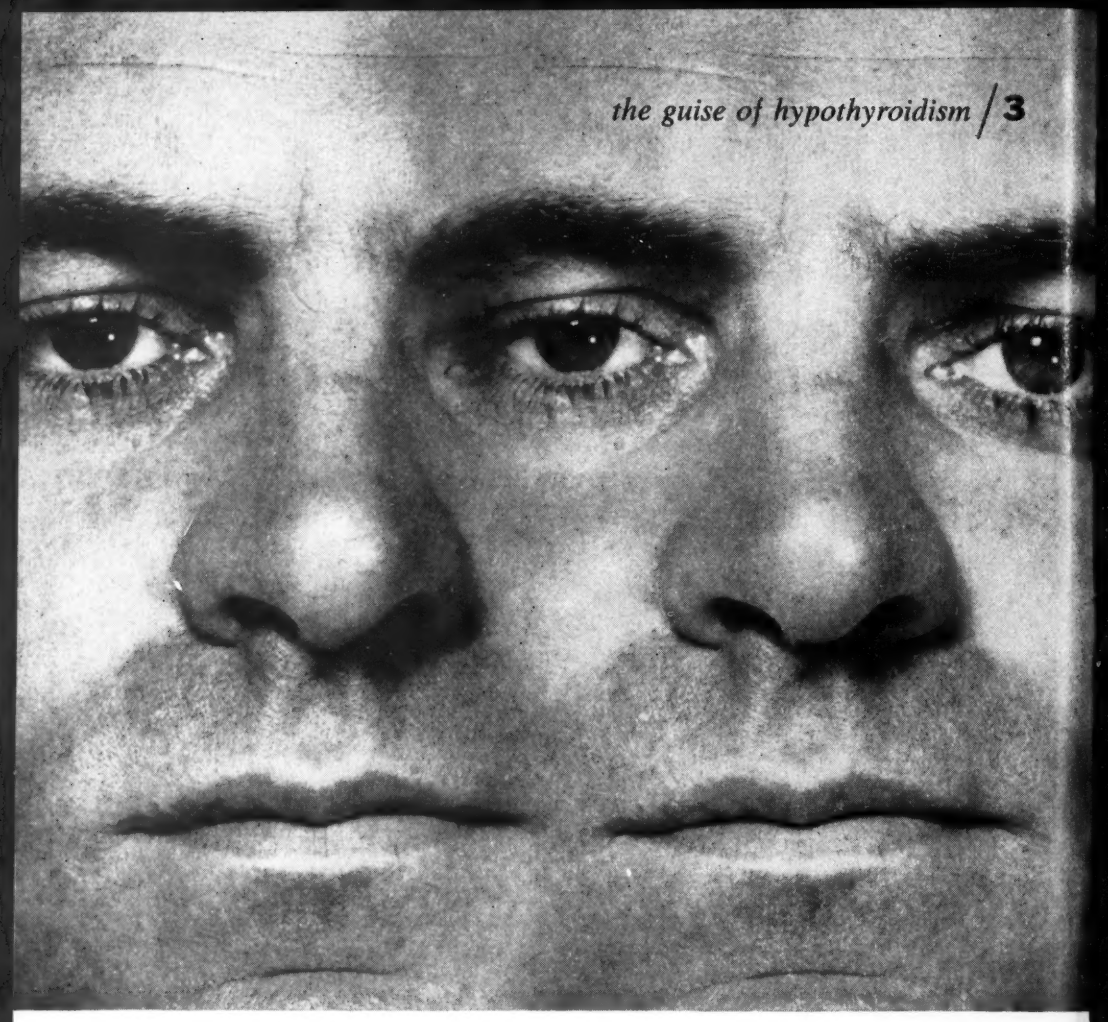

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Presenting symptoms of hypothyroidism are not always clearly defined. "For every patient with clinical myxedema there must be at least a hundred patients with hypothyroidism *sine* myxedema."[1] The many features of this disorder often blur its clinical picture.

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AN UNUSUAL INSTANCE OF THYROXINE-BINDING BY HUMAN SERUM GAMMA GLOBULIN*

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THYROXINE comprises the major portion of the serum iodine in euthyroid and hyperthyroid humans (1–3). Gordon *et al.* (4) and others have demonstrated that most of the serum thyroxine is attached to an alpha globulin, and the remainder is bound to albumin. Little or no thyroxine has been found in association with the beta and gamma globulins.

The present report describes the unusual occurrence of thyroxine-binding by serum gamma globulin in a patient with functioning carcinoma of the thyroid. This was a unique finding among 23 such patients in whom detailed studies of the serum iodine were performed (5).

MATERIALS AND METHODS

The patient was a 26-year-old woman with carcinoma of the thyroid which had metastasized to the cervical lymph nodes and to the lungs. The carcinoma was first detected at age 14, at which time surgical excision of the tumor and partial thyroidectomy had been performed. A similar operation was carried out two years later, because

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of local recurrence. On both occasions, histologic sections revealed papillary carcinoma of the thyroid. Pulmonary metastases, in the form of diffuse miliary lesions, were first detected at age 25. Although the patient was in good general health, her past history was significant in that, at age 4, she had had an enucleation of her left eye because of retinoblastoma. At age 26, a hysterectomy had been performed because of metrorrhagia due to a uterine fibromyoma.

Radioiodine studies were first carried out at age 25, and revealed considerable iodine uptake in the pulmonary metastases, as well as in the remaining normal thryoid tissue. The patient was judged to be euthyroid on the basis of clinical and laboratory evaluation (BMR +16 per cent, serum protein-bound iodine level 4.8 μg. per 100 ml., and serum cholesterol level 157 mg. per 100 ml.). In order to destroy the remaining normal thyroid tissue, the patient was given 75 millicuries of I^{131}-iodide. The urinary excretion of I^{131} totalled 56 per cent of the dose in forty-eight hours, at which time 12 per cent of the dose had been accumulated in the neck. Thyroidal uptake of I^{131} decreased following this treatment, but the patient remained euthyroid due to persistent function in the metastases. Two months after the treatment dose of I^{131}, administration of Tapazole (methyl mercaptoimidazole) was started. This resulted in the development of clinical myxedema (BMR −27 per cent, serum protein-bound iodine level 0.7 μg. per 100 ml., and serum cholesterol level 280 mg. per 100 ml.) and an increased avidity of the pulmonary metastases for iodine. Five months after the institution of Tapazole therapy, and four days after stopping the drug, the patient was given 249 millicuries of I^{131}-iodine. The urinary excretion of I^{131} in forty-eight hours totalled 30 per cent of the dose. X-ray examination of the chest subsequently revealed a decrease in the pulmonary metastases, but three months after the dose, intractable respiratory failure developed, and the patient expired after a terminal illness which lasted for a month. Autopsy revealed extensive radiation pneumonitis, as well as widespread follicular metastases in the lungs.

The serum radioiodine was studied on the fourth and sixth days following the 75-millicurie dose, at intervals from one to twenty-nine days after the 249-millicurie dose, and on the fourth day after a 0.5-millicurie dose given just prior to the second treatment. The following techniques were employed: 1) trichloroacetic acid—*n*-butanol extraction, 2) salting out with phosphate buffer, 3) paper chromatography of whole serum in *n*-butanol—2,4-dioxane—ammonia and *n*-butanol-acetic acid—water, 4) paper electrophoresis in barbital buffer, pH 8.6. The details of these procedures have been referred to elsewhere (5). Additional procedures will be described under Results.

Radioiodine in liquid samples was measured in a well-type Geiger-Mueller or scintillation counter. Radioactivity on paper strips was measured with a thin mica-window Geiger-Mueller tube and a continuously recording counting rate meter. The width of the counting aperture was 1 cm.

RESULTS AND DISCUSSION

With the exception of the electrophoretic analyses, the results presented in Table 1 are like those found in other patients with functioning carcinoma of the thyroid (5). Thyroxine was the major organic radioiodine component in each serum sample, but a considerable proportion of I^{131} failed to move from the origin of the chromatogram. The latter material, however, was not the same in all samples. In the sera obtained after the 75-mc. and 0.5-mc. doses, the immobile component appeared to be in the form of "com-

pound X," a substance found in the serum from many patients with functioning carcinoma of the thyroid (5). This interpretation is based upon the relatively high solubility of serum I^{131} in 1.75 M phosphate buffer, the relatively high proportion of I^{131} associated with serum albumin, and the unlikelihood of significant destruction of thyroid tissue by the 0.5-mc. dose.[1] After the 249-mc. dose, however, the striking variation in the proportion of immobile I^{131} was due to thyroglobulin, since this component increased

TABLE 1. ANALYSES OF SERUM RADIOIODINE

Dose of I^{131} (mc.)	Days after dose	Chromatography			Electrophoresis			Soluble in TCA-BuOH (% org. I^{131})	Soluble in 1.75 M PO_4 buffer (% org. I^{131})
		Thyroxine (% org. I^{131})	Origin* (% org. I^{131})	Iodide† (% tot. I^{131})	Albumin (% PBI^{131})	Alpha globulin (% PBI^{131})	Gamma globulin (% PBI^{131})		
75.0	4	60	40	27	38	34	19‡	75	72
	6	55	45	28	26	43	16‡	64	61
0.5	4	70	30	40	—	—	—	82	51
249.0	1	79	14	28	11	54	35	88	59
	5	63	35	13	14	61	25	64	50
	8	52	45	8	13	68	19	63	42
	12	60	38	7	16	64	20	69	57
	20	67	33	8	17	58	25	79	63
	29	71	24	3	24	57	19	83	71

* Radioiodine which was immobile in the chromatographic systems.

† These values were very close to those obtained by calculating the percentage of serum I^{131} soluble in 2.98 M phosphate buffer.

‡ Strip was stained before counting. The remaining I^{131} was in the beta globulin area.

at a time when radiation damage to thyroid tissue would be expected, and since it was accompanied by a decreased solubility of serum I^{131} in both trichloracetic acid—butanol and in 1.75 M phosphate buffer, and an increase of the I^{131} in alpha globulin. These properties are characteristic of thyroglobulin (6, 7).

Triiodothyronine comprised the remainder of the organic I^{131} in serum, except for the presence in a few instances of an unidentified substance amounting to 3 per cent or less of the serum I^{131}. The variations in iodide-I^{131} values after each of the radioiodine doses were apparently related to

[1] The amount of tumor tissue was conservatively estimated to be 200 grams.

differences in the uptake of iodide by thyroid tissue, since there was no evidence for abnormal renal function.

Electrophoretic analsyis of serum I^{131} following the large doses of radioiodine revealed, in addition to the expected peaks in the albumin and alpha-globulin areas, an unusual quantity of radioiodine associated with gamma globulin. The electrophoretic pattern of serum I^{131} on the first day

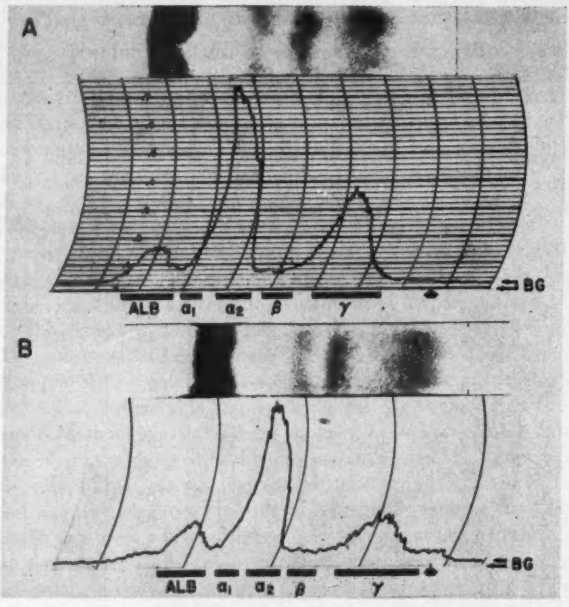

FIG. 1. Zone electrophoresis of serum at pH 8.6. (Barbital, ionic strength 0.1.) **A.** Serum obtained one day after the 249-mc. dose of I^{131}. **B.** Non-radioactive serum obtained prior to the 249-mc. dose, to which I^{131}-labeled *l*-thyroxine had been added. The paper strips, stained with bromophenol blue, are aligned with records of their radioactivity. The single arrow denotes the point of application of the serum. BG indicates background radioactivity.

after the 249-mc. dose is presented in Figure 1A. Although this serum contained the largest gamma globulin-I^{131} component, a similarly well defined peak of radioiodine was present in gamma globulin in all sera tested in this patient. Variations in the proportion of I^{131} in gamma globulin were apparently related to variations in the relative concentrations of compound X and thyroglobulin in each of the sera, since the latter compounds do not become associated with gamma globulin. The alignment of the I^{131} with the more rapidly moving gamma globulin was a consistent finding in all sera.

A portion of the gamma globulin in serum obtained five days after the 249-mc. dose was separated from the other proteins by moving boundary electrophoresis in barbital buffer, pH 8.6, and was removed from the descending limb of the electrophoretic cell with a long needle.[2] A mixture of the isolated gamma globulin and Cohn fraction II of human plasma[3]

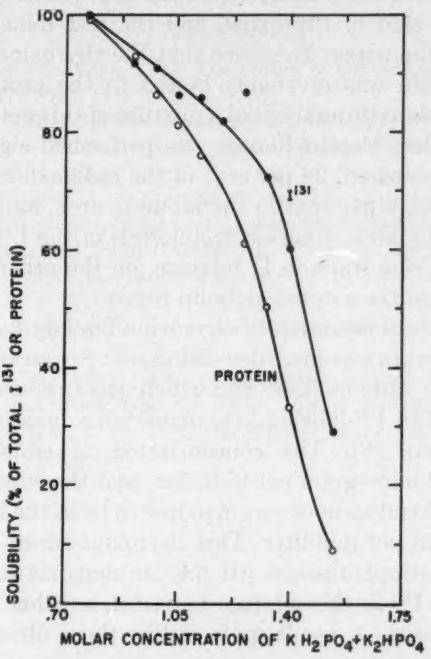

FIG. 2. Salting out in phosphate buffer (equimolar KH_2PO_4 and K_2HPO_4, pH 6.5) of a mixture of isolated gamma globulin from the patient's serum and Cohn fraction II of human plasma. Protein was determined by biuret analysis (6).

was prepared, and subjected to salting out with phosphate buffer. The mixture was such that the final solutions contained, in addition to the appropriate buffer concentration, approximately .01 ml. of the isolated gamma globulin and 2 mg. of Cohn fraction II. Although the protein content of the isolated gamma globulin solution was not measured, it was estimated that the final solution contained less than .02 mg. of the pa-

[2] The authors wish to thank Dr. Mary L. Petermann of the Sloan-Kettering Institute for this preparation.

[3] Cohn fraction II of human plasma was obtained through the courtesy of Dr. S. T. Gibson of the American National Red Cross and Dr. J. N. Ashworth of E. R. Squibb and Sons. At least 98 per cent of it was gamma globulin.

tient's gamma globulin. Salting-out curves for radioiodine and protein in this mixture are presented in Figure 2. It is evident that the I^{131} was slightly more soluble than the average gamma globulin.

The I^{131} isolated with the gamma globulin was also subjected to paper chromatography in the butanol—acetic acid and butanol—dioxane—ammonia systems. In these analyses, 88 per cent of the radioiodine had an Rf identical with that of thyroxine, and the rest remained at the point of application to the paper. Evidence that the thyroxine-I^{131} isolated with the gamma globulin was reversibly bound to the protein was obtained from zone electrophoretic analysis of a mixture of this material with normal human serum. When electrophoresis was performed eighteen hours after the mixture was prepared, 28 per cent of the radioiodine was found in the alpha globulin area, 6 per cent in the albumin area, and the remainder in the gamma globulin area. Zone electrophoresis of the I^{131} isolated with the gamma globulin-Cohn fraction II mixture, on the other hand, revealed a single peak of I^{131} in the gamma globulin region.

A more direct demonstration of thyroxine-binding by gamma globulin in this patient's serum was provided as follows: Serum which had been obtained prior to the 249-mc. dose, and which was free of endogenous radioiodine, was added to I^{131}-labeled *l*-thyroxine[4] in a manner which has been described elsewhere (8). The concentration of added thyroxine was approximately .02 microgram per milliliter, and the endogenous thyroxine level (assuming all endogenous serum iodine to be in the form of thyroxine) was .01 microgram per milliliter. This thyroxine-serum mixture was subjected to zone electrophoresis at pH 8.6. As demonstrated in Figure 1B, 28 per cent of the I^{131} in this mixture moved in a rather sharp band in the gamma globulin area—a result quite unlike those obtained with normal serum (8).

The serum proteins in this patient did not appear grossly different from those of normal serum with respect to electrophoretic pattern (visual inspection of the stained paper strips) or salting-out curve in phosphate buffer. The serum total protein concentration was 6.7 Gm. per 100 ml.

SUMMARY AND CONCLUSIONS

The patient described in this report was unique among other patients with carcinoma of the thyroid, as well as among normal and hyperthyroid subjects, in that the serum consistently contained a large proportion of its iodine in a form associated with gamma globulin. It has been demonstrated that this abnormality was due to the unusual occurrence of thyroxine-binding by serum gamma globulin, rather than to the presence

[4] I^{131}-labeled *l*-thyroxine was supplied by Abbott Laboratories.

of any unusual iodine-containing compound. Clinical data did not single out this case from other cases of functional carcinoma of the thyroid, and the significance of this abnormality remains obscure.

Acknowledgment

The authors wish to thank Miss Helen Hagopian for technical assistance.

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EFFECT OF EXOGENOUS CORTICOTROPIN ON THE HISTOCHEMICAL PATTERN OF THE HUMAN ADRENAL CORTEX AND A COMPARISON WITH THE CHANGES DURING STRESS

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INTRODUCTION

THE histologic changes which occur in the human adrenal cortex in conditions of stress are attributed to the liberation of endogenous corticotropin (ACTH). The effect of exogenous corticotropin on the adrenal gland is well known in animals, but only recently has it been possible to study the changes which take place in the human adrenal cortex as a result of such stimulation. Variations occur in lipid pattern, cytology, enzyme content and ribonucleic acid content of the cells. A comparison of the changes during stress with those found during administration of corticotropin forms the basis of this paper.

MATERIALS

Adrenal glands were received from two groups, 1) cases of mammary and prostatic carcinoma treated by bilateral adrenalectomy, and 2)postmortem cases in which the patients were severely stressed until the time of death.

Group 1 (15 cases). The glands in this group were obtained from two-stage adrenalectomies. The first gland, which was removed from the patient before any hormonal (corticotropin or cortisone) treatment, served as a control; the second gland was removed after an interval of approximately three weeks. The patients received 100 mg. of corticotropin (as ACTHAR Gel) daily for four days prior to the second operation, to stimulate endogenous corticosteroid production. The direct effect of corticotropin on the adrenal cortex could, therefore, be studied and the changes in each case compared with those in the first or control gland. The process of ACTH administration was reversed in one patient in order to determine the stress effect of the operation. She received 100 mg. daily for four days before the first adrenalectomy and the second gland was removed after the usual three-week interval. As it was not considered justifiable to remove the latter adrenal without some supportive hormone therapy, cortisone (100 mg.) was given twenty-four hours before the second operation. The second adrenal was normal histologically in all respects. It was of normal size and not hypertrophied, as might have been expected in the course of compensating for the loss of the first adrenal.

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Group 2 (50 cases). All postmortem examinations were performed within four hours of death. Only those cases were included in which a severe stress was known to have been active and in which death occurred within a period of eight days following the stress—mainly severe burns, coronary thrombosis, various complications following elective surgery, and infections such as acute generalized peritonitis, lobar pneumonia, gastroenteritis and bacillary dysentery.

METHODS

Thin adrenal slices were fixed in 10 per cent neutral formol-calcium solution; some were embedded in gelatine, others in paraffin. One slice was fixed in cold acetone at 4° C. and then embedded in paraffin. Sections of gelatine-embedded material cut at 11μ were stained by hematoxylin and Sudan IV for adrenal lipids; unstained sections were mounted in glycerol jelly and examined under polarized light for bi-refringent crystals. Cholesterol and cholesterol esters were stained by the Schultz reaction (1, 2). Paraffin sections cut at 6μ were stained with hematoxylin and eosin and used for cytologic examination. Adjacent sections from the same block were stained by pyronin-methyl green for ribonucleic acid (RNA), with or without prior treatment with ribonuclease to remove the RNA (3). Paraffin sections cut at 6μ after fixation in cold acetone were used for most of the enzyme studies. Both the azo-coupling (4) and the Gomori glycerophosphate method as described by Pearse (5) were used for alkaline phosphatase. Cysteine in 0.0025 M concentration was employed as a control inhibitor in both methods (6). The Gomori technique was used for acid phosphatase (7). Control sections were incubated in the buffer solution containing the lead salt but without substrate, to eliminate the possibility of nonspecific precipitation. The inhibitory effect of 0.01 M sodium fluoride was examined, as well as the effect of cysteine, when added to the incubation fluid. The Gomori method for alkaline-phosphatase was adapted for adenosinetriphosphatase. Adenosinetriphosphate as the sodium salt was used instead of Na-β-glycerophosphate as the substrate. The micro method described by Glick and Fischer (8) was also used and found to be satisfactory. Since adenosinetriphosphatase is a sulphydryl dependent enzyme, control sections were treated by a substrate to which cysteine (0.0025 M concentration) was added. Further sections were treated with the substrate to which Salyrgan (0.1 ml. per 10 ml.) was added; this acts as an inhibitor of adenosinetriphosphatase (6). The azo-coupling method was employed for nonspecific esterase, using naphthol AS acetate as substrate (9).

The activity of the dehydrogenase enzymes was examined by a modification of the techniques described by Pearse (10). Slices (1–3 mm.) from all adrenals removed at operation, and from some postmortem adrenals removed within two hours of death, were incubated for two hours at pH 7.5 in a phosphate buffer solution containing the sodium salts of the citric-acid cycle intermediates (succinic, citric and malic acids) in 0.1 M concentrations. A 1 per cent solution of triphenyltetrazolium chloride (TTC) was used as the hydrogen acceptor in the incubation fluid. After incubation the slices were fixed in 10 per cent neutral formalin for fifteen minutes, and frozen sections were cut and mounted in glycerol jelly. Sodium malonate was used to inhibit succinic dehydrogenase at an inhibitor/substrate concentration ratio of 5:1, and was incorporated in control solutions when succinate, citrate and malate were used as substrate.

RESULTS

Attention was directed to the following points when assessing the effect of administration of corticotropin on the adrenal cortex: cytology, altera-

tion in lipid and cholesterol content of the glands, and in the ribonucleic acid and enzyme systems. These factors will be considered first in the normal control glands and then in the ACTH-stimulated glands of the two-stage bilateral adrenalectomy of Group 1 in this series, and the changes compared with those seen on Group 2.

Group 1. Adrenals from bilateral adrenalectomies

Cytology

The three zones (glomerulosa, fasciculata and reticularis) are marked clearly in the normal gland (Fig. 1a). The cells in the zona reticularis are small and compact in type, and pass abruptly into the zona fasciculata, where they are large, have a clear cytoplasm (Fig. 2a) and are arranged in regular, prominent columns. A thin layer on the surface forms the zona glomerulosa, in which the cells are small in size and show only an ill-defined cell border. Their nuclei are more hyperchromatic than in the cells of the other two zones.

After 400 mg. of corticotropin, a distinct transformation occurs. Differentiation of the cortex into fasciculata and reticularis is lost and the two zones appear as a homogeneous layer which extends almost, but not quite to the zona glomerulosa (Fig. 1b). The cells are hypertrophied, have a fleshy appearance, stain deeply with eosin and have small bluish basophilic granules (ribonucleic acid or ribonucleoprotein) scattered throughout the cytoplasm. They are called compact cells (11) (Fig. 2b). Clear cells are found only at the junction of this zone with the zona glomerulosa. The latter appears rather compressed and shows no significant alteration in cell type.

Lipid and cholesterol (free and esterified)

The normal gland contains abundant lipid and cholesterol deposits in all zones. These substances appear as fine droplets in the zona reticularis and zona glomerulosa, and as large globules in the cells of the zona fasciculata (Fig. 3a). After administration of corticotropin, the most striking feature is the marked uniform depletion of lipid and cholesterol from the gland (Fig. 3b). All three zones are affected and whatever lipid remains is in the form of small or medium-sized droplets.

Ribonucleic acid (RNA)

This substance is concentrated in the cytoplasm of the cells of the zona reticularis and the zona glomerulosa in the normal gland; there is no stainable deposit in the zona fasciculata (Fig. 4a). After administration of corticotropin, abundant small granules staining for RNA appear in the cytoplasm of the fleshy compact cells which now constitute the major part

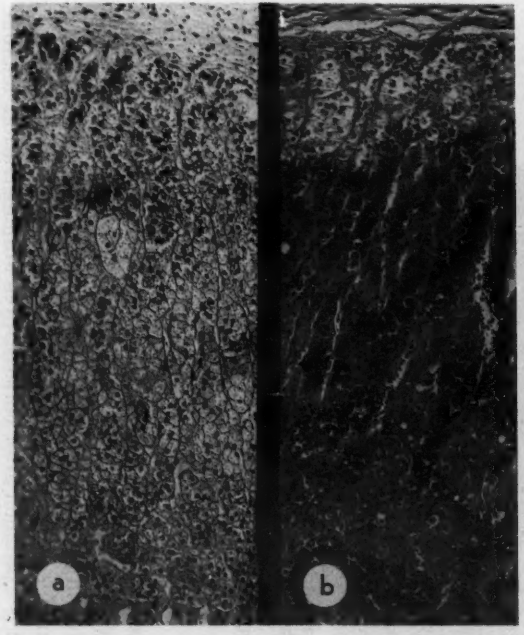

FIG. 1

FIG. 2

FIG. 1. The normal or control adrenal is shown on the left and the corticotropin-treated gland on the right. The zona reticularis is distinctly separated from the zona fasciculata in the normal gland (1**a**), but both zones appear united after administration of corticotropin (1**b**). (H. & E. ×100)

FIG. 2. The zona fasciculata is composed of large clear cells arranged in columns (2**a**). This vacuolated appearance is lost after corticotropin treatment (2**b**); the cytoplasm of the cells becomes eosinophilic and slightly granular (compact cells). (H. & E. ×250)

of the adrenal cortex (Fig. 4b). The cells of the zona glomerulosa likewise stain strongly for RNA.

Enzymes

Acid and alkaline phosphatase and nonspecific esterase are localized strictly to the zona reticularis of the normal gland (Figs. 5a and 6a). The cells of the zona fasciculata and glomerulosa are negative; only the base-

FIG. 3. The distribution of lipid in the normal gland is shown in 3a. After stimulation with corticotropin, lipid depletion occurs in all zones (3b). (H. & Sudan IV ×250)

ment membrane of the vessels in both zones stains for alkaline and acid phosphatase. Succinic, iso-citric and malic dehydrogenase are in most instances distributed in the zona reticularis and glomerulosa; the reaction is negative in the zona fasciculata (Fig. 7a).

When the gland is examined after corticotropin administration, alkaline and acid phosphatase are present in the compact cells which occupy most of the cortex and extend almost to the zona glomerulosa (Figs. 5b and 6b). Those cells are rich also in succinic, iso-citric and to a lesser extent malic dehydrogenase (Figs. 7b and 8). Corticotropin appears to have little effect

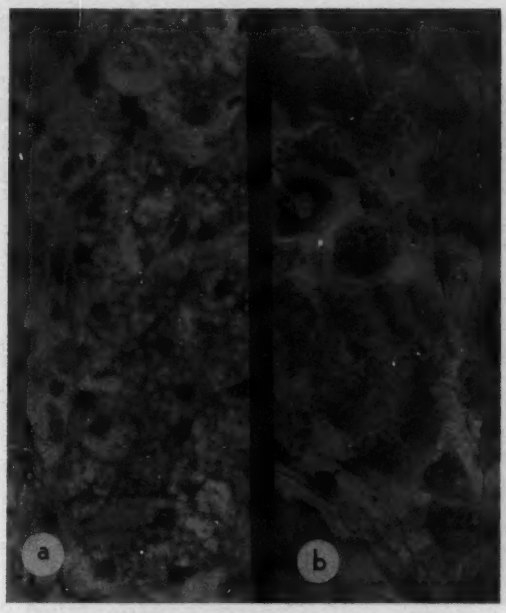

FIG. 4. The large clear cells of the zona fasciculata are poor in stainable RNA (4a). In contrast, small discrete granules appear in the cytoplasm of the compact cells following administration of corticotropin (4b). (Pyronin/methyl green ×700)

on the distribution of nonspecific esterase, as shown by this technique; the enzyme remains localized, as in the normal gland, to the zona reticularis. No specific adenosinetriphosphatase could be detected in the normal or in the corticotropin-treated gland.

Group 2. Postmortem adrenals from patients severely stressed until the time of death

The appearance of the adrenal cortex in this group conforms to one of two patterns which are based mainly on the degree of lipid depletion (11):

1. Focal lipid depletion.
2. Extreme diffuse lipid depletion.

Focal lipid depletion

Focal lipid depletion of the adrenal cortex was observed in fatal adult burns as early as four hours and as late as eight days after the injury. It was seen eight hours after a fatal attack of coronary thrombosis, and is

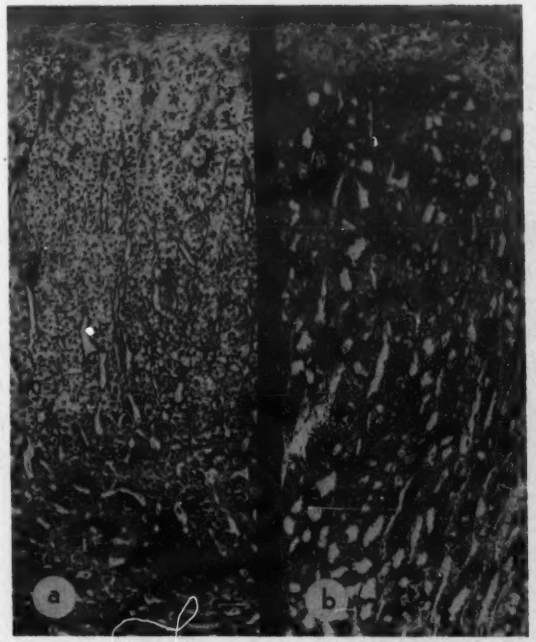

FIG. 5

FIG. 6

FIG. 5. The localization of alkaline phosphatase is shown in the zona reticularis of the normal gland (5a). The rest of the gland shows no enzyme, but the basement membrane of the capillaries is stained. After corticotropin (5b), alkaline phosphatase appears in most of the cells of the zona fasciculata. A negative area is present on the surface of this zone; the zona glomerulosa is deficient in this enzyme. (Alkaline phosphatase ×100; Mannheimer and Seligman's azo-coupling)

FIG. 6. The distribution of acid phosphatase is shown in the normal (6a) and in the corticotropin-treated gland (6b). It is similar to that of alkaline phosphatase. A small deposit of enzyme is present in the cells of the zona reticularis in the normal gland (bottom left, 6a), whereas a marked deposit is seen after administration of corticotropin (6b). Note also in the latter figure the small zone of clear cells in the upper zona fasciculata, from which the enzyme is absent. The zona glomerulosa is shown between this zone and the overlying capsule. (Acid phosphatase ×150; Gomori)

the commonest adrenal pattern observed in death from conditions of stress. It can be seen in a modified form in children twenty-four to forty-eight hours after a severe burn, and occurs following a fatal attack of gastro-enteritis. It represents a wide range, from small focal areas to almost complete depletion of the gland.

In the normal part of the gland between the areas of focal lipid deple-

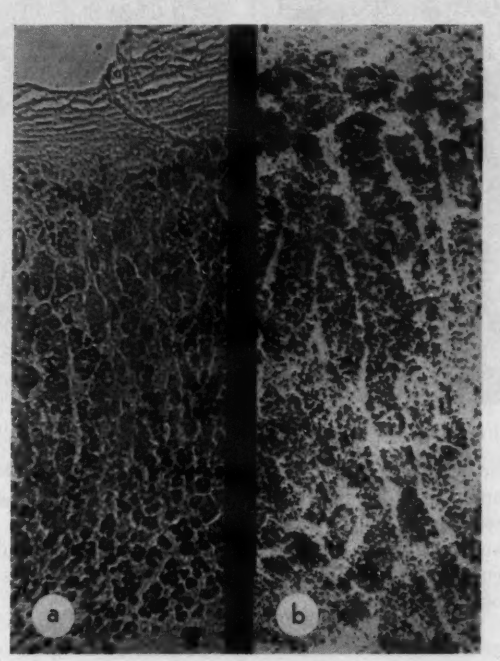

FIG. 7. Dehydrogenase activity is present in the zona reticularis and zona glomerulosa of the normal gland (**7a**). The activity extends throughout all the zones after administration of corticotropin (**7b**). (Succinic dehydrogenase ×120)

tion, the cells are clear in type and contain large lipid and cholesterol globules. They are poor in enzymes and in RNA. On the other hand, the patchy lipid-depleted areas are composed of compact cells, rich in RNA and enzymes. The cells may be completely free of lipid or contain only fine globules.

Extreme diffuse lipid depletion

Extreme diffuse lipid depletion of the adrenal cortex was found as early as the fourth day in the adrenals of severely burned children. It is not a

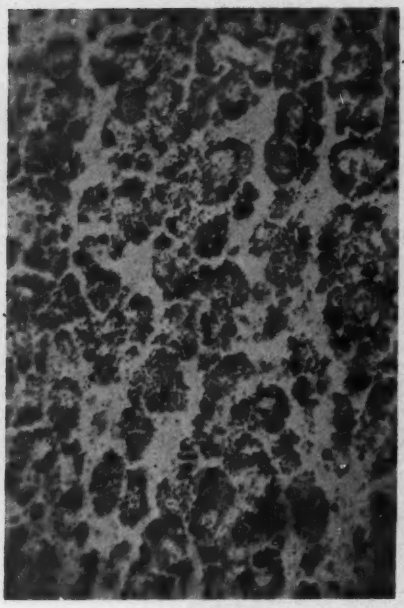

FIG. 8

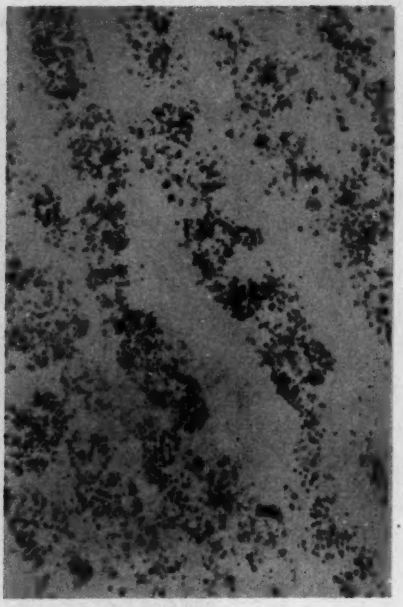

FIG. 9

FIG. 8. Iso-citric dehydrogenase in the cells of zona fasciculata following administration of corticotropin. Note the medium sized deposits and compare with Figure 9. (Iso-citric dehydrogenase ×250)

FIG. 9. The gland in this case contains very little lipid and so the deposit of reduced dye following incubation with TTC and sodium succinate consists of fine granules. (Succinic dehydrogenase ×250)

common picture in the adult adrenal, but was seen in severe burns after six or seven days, as well as in infections, particularly when death was due to peritonitis. It resembles closely the adrenal pattern observed following administration of corticotropin. There is no differentiation into zona reticularis and zona fasciculata. The cortex is composed of compact cells extending up to the zona glomerulosa, which appears compressed. These cells are rich in enzyme systems (Fig. 9) and RNA but poor or deficient in stainable lipids and cholesterol.

DISCUSSION

The adrenal glands from two-stage bilateral adrenalectomy cases (Group 1) are most satisfactory for studying the effects of corticotropin, since the first gland (removed from the patient at a time when there has been no hormonal treatment) acts as a control. Postmortem adrenals (Group 2) are satisfactory when a stress is known to have been active until

the time of death (*e.g.*, burns, coronary thrombosis or traumatic injury) (11). The reaction of the human adrenal cortex to exogenous corticotropin (Group 1) is similar to that found in patients dying from conditions of stress (Group 2) and so confirms the belief that the adrenal changes in stress are due to the action of endogenous corticotropin. There is an increase in the weight of the gland and hypertrophy of individual cells of the zona reticularis and zona fasciculata (12). This results in an increase in the width of the cortex (13–15). There is a well marked diminution of unsaturated lipids, cholesterol and cholesterol esters in the gland. This may occur in adults in a rather discrete focal manner, the extent of depletion depending on the severity and duration of the stress. The lipid depletion is diffuse in children and in very severe stress in adults, and is similar to that induced in this series by exogenous corticotropin.

The cells of the zona fasciculata undergo a striking change with the loss of lipids. They become compact in appearance, their cytoplasm stains intensely eosinophilic, and numerous small basophilic granules containing RNA appear prominently in their cytoplasm. This type of cell may be devoid of lipid, but when the latter is present it occurs either in medium sized or fine globules. The compact cell is rich in alkaline and acid phosphatase as well as in the dehydrogenase enzyme systems of the citric-acid cycle. It has been noted already that similar, though smaller, compact cells occur normally in the zona reticularis. As a result of the formation of compact cells in the zona fasciculata, differentiation between the two zones is lost and the adrenal cortex, exclusive of zona glomerulosa, presents a uniform appearance.

The presence of those active compact cells, rich in enzymes and RNA, in the zona reticularis does not substantiate the belief, widely held, that this is a senescent zone. Likewise, the gradual extension outwards (Figs. 5b and 6b) and the transition from clear to compact cells (Figs. 2a and 2b) under the influence of corticotropin, is an indication of the erroneous nature of the theory of centripetal movement (16, 17), whereby cells arise under the capsule, mature and form their secretion in the zona fasciculata and die in the zona reticularis or senescent zone. On the contrary, our observations would appear to indicate that the zona reticularis may be the site of corticosteroid production in the normal gland. Under the action of either endogenous or exogenous corticotropin, alteration of the cells from clear to compact type occurs from within outwards and results in an increase in corticosteroid production. In conditions of stress in adults, this occurs in a focal manner but in children and after administration of corticotropin, a more diffuse depletion occurs. This difference in the mode of lipid depletion in adults in conditions of stress and after exogenous corticotropin is not easily explained at present.

As the changes found in the adrenocortical cells after stress and exogenous corticotropin are associated with the formation of numerous cortical hormones, of which 17-OH-corticosterone (cortisol) and corticosterone are the principal components (18, 19), it can be deduced that the compact cells are engaged in the formation and secretion of these hormones.

Before considering the part played in steroid synthesis by the many substances studied histochemically in this investigation, it is well to reflect on the specificity of the methods used to demonstrate them.

Lipids and cholesterol

Sudan IV as an adrenal stain is widely used and is entirely satisfactory in demonstrating changes in lipid pattern. Recent observations (20–22) indicate that histochemical ketosteroid methods (2:4, dinitrophenylhydrazine, azo-coupling (23)) stain no more than unsaturated lipids and cholesterol. This is in agreement with the view expressed by Rogers and Williams (24) that very little preformed steroid is present in the adrenal cortex. The Schultz reaction appears to be specific for cholesterol and cholesterol esters. Kent (25) found that only β-carotene gave a comparable result.

Ribonucleic acid

The pyronin-methyl green technique as used by Brachet (3) can be regarded as specific for ribonucleic acid, especially when test and control sections are employed, with and without ribonuclease, respectively. The presence of RNA in the adrenal cortex in this investigation has been confirmed by chemical analysis (12).

Enzymes

Alkaline phosphatase. Nuclear deposits were present in all cells (clear and compact) in the glycero-phosphate method (Gomori) but not in the azo-coupling technique (Manheimer and Seligman). Otherwise, comparable results were obtained by both methods. When cysteine was introduced into the incubating fluid, the reaction with both techniques was inhibited (Figs. 10a and 10b). Similarly, the positive result obtained when ATP was used as the substrate in the Gomori technique, was inhibited and not increased by cysteine. Since adenosinetriphophatase is a sulphydryl dependent enzyme and alkaline phosphatase (phosphomonoesterase) is inhibited by the sulphydryl-containing amino acid, cysteine, it can be concluded that the positive result with ATP does not represent a specific adenosinetriphosphatase but is due to hydrolysis of ATP by a nonspecific alkaline phosphatase, as has been shown with other tissues (26).

Acid phosphatase. Patchy distribution of the stain and nonspecific de-

posit of lead salts are two objections raised against the original technique (27). Observations by Newman, Kabat and Wolf (28) indicate that nonspecific deposit of lead salts occurs infrequently if the pH of the solution is maintained below 5, and the patchy distribution of staining can be prevented by increasing the concentration of substrate (7). A buffer substrate ratio of 5:1 was found to be optimum. This Gomori technique was used in the present investigation and, although uniform pictures were usually obtained, patchy patterns were occasionally found. Control sections in-

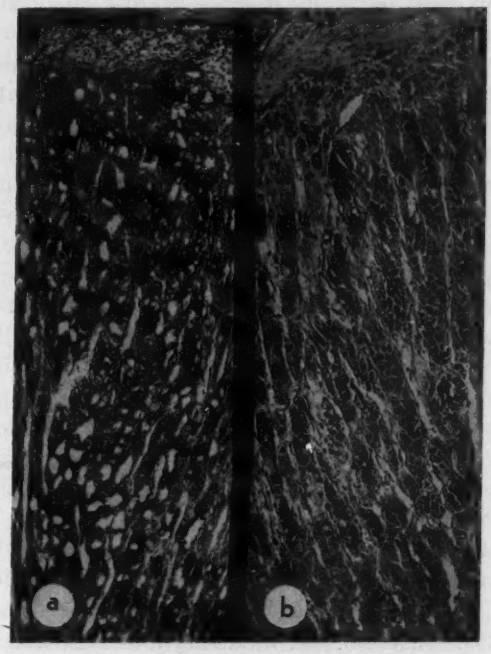

FIG. 10. The distribution of alkaline phosphatase in the adrenal cortex following administration of corticotropin is shown in 10**a**. When a solution of 0.0025 M concentration of cysteine is incorporated in the medium, there is a negative reaction (10**b**). (Manheimer and Seligman ×100)

cubated in a buffer solution containing lead salts without substrate eliminated the effects of nonspecific precipitation, and the inhibitory effect of 0.01 M sodium fluoride was demonstrated. Cysteine when added to the incubation medium at a concentration of 0.025 M did not inhibit the reaction, as was expected; acid phosphatase, unlike alkaline phosphatase, is not inhibited by sulphydryl groups. There is no doubt that with the Gomori technique, a definite increase in the deposit of lead salts occurs in the adrenocortical cells in stress and after administration of corticotropin.

Dehydrogenase. Our attention was directed to the study of the dehydrogenases in the adrenal cortex as a result of the observations of Brownie and Grant (29), who showed that citric-acid cycle intermediates were necessary for the 11 β-hydroxylation of desoxycorticosterone by isolated ox adrenocortical mitochondria. Figure 11 illustrates a simplified form of the citric-acid cycle and demonstrates the substrates on which the different dehydrogenases act. Since only TTC, of the tetrazolium compounds, was

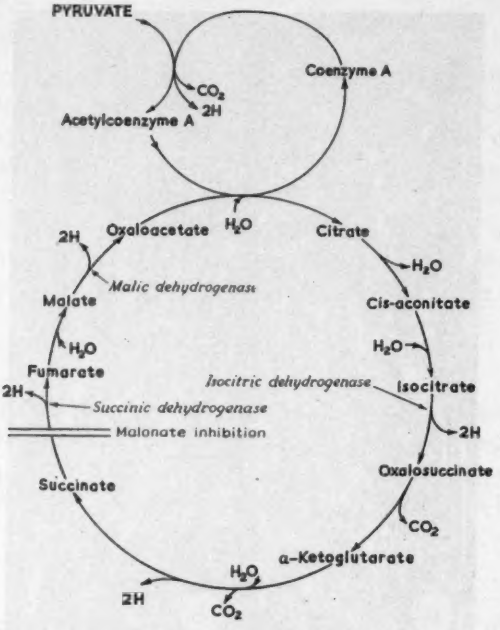

FIG. 11. A simplified form of the citric-acid cycle demonstrating the substrates on which the different dehydrogenase enzymes act.

available to us, it was necessary to use thin adrenal slices approximately 1 to 3 mm. in thickness. When sodium succinate was introduced into the phosphate buffer solution (pH 7.5) and incubated with adrenal slices, the positive purple color obtained with corticotropin-treated adrenals could be inhibited if sodium malonate were added in the proportion of 5 parts to 1 of succinate. This result indicates the presence of succinic dehydrogenase, which oxidizes succinate to fumarate with the loss of two hydrogen atoms which are taken up by the TTC to produce the change in color. Malonate is a competitive inhibitor of this enzyme, and when it is added to the

substrate the negative reaction resulting is further proof of the presence of succinic dehydrogenase. As iso-citrate was not available as a substrate, sodium citrate was used with the assumption that this would be converted to iso-citrate by aconitase in the slice. Sodium malonate was added once more and the effect of succinic dehydrogenase eliminated. The very strong reaction illustrated in Figure 8 demonstrates the reaction of iso-citric dehydrogenase. The presence of malic dehydrogenase was shown also, but the reaction was always much weaker than that for other enzymes. The results obtained with adrenal slices in this series when different substrates were used, with and without malonate as an inhibitor, indicate that in the adrenocortical cells of corticotropin-treated patients we are dealing with increased activity of citric-acid cycle dehydrogenases. It should be noted that only a very weak reaction was obtained with TTC, when the adrenal slices were incubated in the phosphate buffer without any substrate. The weak reaction can be attributed to the presence of a low-normal concentration of citric-acid cycle intermediates within the adrenal cells. This result indicates that the positive reactions with succinate, citrate and malate are not due to spontaneous reduction of TTC by lipid— one of the defects attributed to this substance (30).

When TTC, a colorless compound, is reduced, it forms a red substance (formazan) (5, 31), but further reduction results in a dark purple deposit. In the present investigation a dark purple color was formed in all instances when the result was positive. The reduced TTC is easily soluble in adrenal lipids and so the resulting picture varies with the size of the lipid droplets. When the lipid is absent or in minute globules, the reduced TTC appears as small dark purple specks (Fig. 9); when the globules are of medium size, the reduced dye dissolves in them and the globules appear purple (Fig. 8); large lipid globules occur only in the clear cells of the normal gland and in every instance they stain a pale pink color. Accordingly, it can be stated that lipid by itself will not reduce TTC and, as far as the adrenal cortex is concerned, reduction of TTC indicates dehydrogenase activity.

CORRELATION OF HISTOCHEMICAL FINDINGS WITH *in vitro* OBSERVATIONS ON CORTICOSTEROID BIOGENESIS

In assessing the part played by cholesterol, enzymes and RNA in corticosteroid formation, reference is made to the tentative scheme of steroid biosynthesis proposed by Hechter (32). This is based on the results of experiments with perfused, sliced or homogenized ox adrenals (Fig. 12).

It has been shown by several workers (33–35), with the aid of radioactive isotopes, that cholesterol and acetate may serve as precursors for the hormones corticosterone (Kendall's compound B) and 17-OH-corticosterone

(Kendall's compound F, or cortisol) formed by the adrenal gland, and that the formation of these substances may be stimulated by corticotropin. Furthermore it has been shown that cholesterol may be synthesized from acetate (36). Of the intermediate pathways from acetate and cholesterol to the hormones, much is unknown, particularly in the human gland. When these findings are considered in the light of our present histochemical observations, the significance of cholesterol depletion in the gland after corticotropin and stress becomes clearer. The normal resting gland is rich

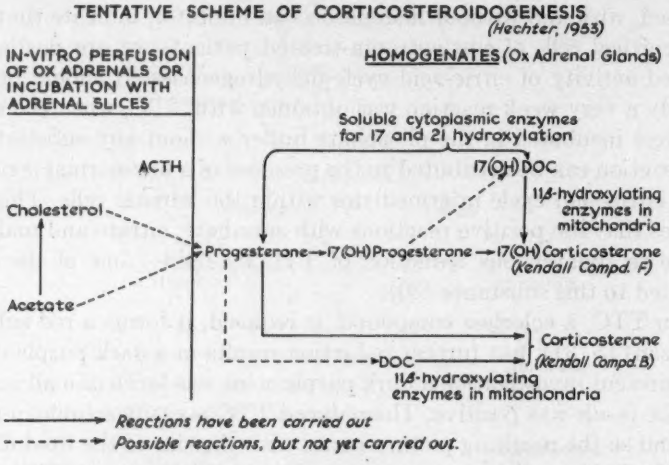
TENTATIVE SCHEME OF CORTICOSTEROIDOGENESIS
(Hechter, 1953)
IN-VITRO PERFUSION OF OX ADRENALS OR INCUBATION WITH ADRENAL SLICES
HOMOGENATES (Ox Adrenal Glands)
Soluble cytoplasmic enzymes for 17 and 21 hydroxylation
ACTH
17(OH) DOC
11β-hydroxylating enzymes in mitochondria
Cholesterol
Progesterone → 17(OH) Progesterone → 17(OH) Corticosterone (Kendall Compd. F)
Acetate
Corticosterone (Kendall Compd. B)
DOC
11β-hydroxylating enzymes in mitochondria
Reactions have been carried out
Possible reactions, but not yet carried out.

FIG. 12. Tentative scheme of corticosteroidogenesis (Adapted from Hechter (32).)

in cholesterol and, when it is stimulated, the conversion of this substance to compound F is brought about with varying degrees of cholesterol and lipid depletion. When the gland becomes depleted of lipid, steroid can still be produced, since the pathway via acetate synthesis may become available. Thus a dual mechanism may exist for the production of corticosteroids. Since cholesterol can be synthesized from acetate (36), a mechanism exists whereby the gland can restock, and it is tempting to suggest that this is indicated by the reversion pattern (11, 37). It should be appreciated, however, that the conversion of acetate to compound F does not necessarily proceed by way of cholesterol as an intermediate product.

The scheme of adrenocortical steroid biogenesis proposed by Hechter *et al.* (35) suggests that progesterone is a key intermediate. This substance is initially hydroxylated at the 17 and 21 positions by enzymes which require ATP and DPN or TPN as well as citric-acid cycle intermediates (38). Brownie and Grant (29) have shown that the citric-acid cycle inter-

mediates are required for the final hydroxylation at the 11-position. It is appreciated that although this scheme of corticosteroid synthesis from progesterone may occur *in vitro*, it does not necessarily represent the usual *in vivo* pathway. Nevertheless it may throw some light on the role of the dehydrogenases observed in the present histochemical investigation. These enzymes appear to play an enhanced part, at least in the adrenal cell, during 11 β-hydroxylation which results in the formation of corticosterone and 17-OH-corticosterone.

There is still the question of the role of alkaline and acid phosphatase and cytoplasmic RNA, all of which are greatly increased in the adrenal cell when it is stimulated by corticotropin.

It has been suggested that alkaline phosphatase plays a part in the absorption of glucose by cells of the renal tubules (39), but there is as yet no direct evidence to indicate what role it plays in corticosteroid formation and secretion. However, it is significant that both phosphatase enzymes, in the normal gland as well as in conditions of stress and after corticotropin, are localized in the zona reticularis and zona fasciculata, but not in the zona glomerulosa. Likewise, the role of RNA is obscure, but this is discussed in more detail elsewhere (12).

The action of corticotropin on corticosteroidogenesis. Since adrenal homogenates without the help of corticotropin can convert progesterone to 17-OH-corticosterone and corticosterone, it has been suggested that corticotropin plays no part in this phase of steroid synthesis (32). The yield of these two steroids increases greatly, however, in perfusion experiments with cholesterol or acetate when corticotropin is added to the substrate, and Hechter (32) has postulated that the action of corticotropin is to break down the side chain of cholesterol (C27) to form a C21 compound, or to build up acetate (or similar C2 substance) into a C21 steroid. The exact mechanism by which this is brought about is obscure. The present investigation merely indicates the alteration of the histochemistry of the adrenocortical cells and the increase in enzyme systems which occur after administration of corticotropin.

SUMMARY

1. Adrenal glands were received from two distinct groups: (a) 15 cases of bilateral adrenalectomy for mammary or prostatic carcinoma, and (b) 50 postmortem cases in which the patients were severely stressed until the time of death.

2. The adrenals removed at operation were received in two stages. The first gland was removed without the patient having had any supportive hormonal therapy (corticotropin or cortisone), and served as a control.

The second gland was obtained after an interval of three weeks. Patients received 100 mg. of corticotropin (as ACTHAR Gel) daily for four days prior to the second operation.

3. The direct effect of corticotropin on the adrenal cortex was observed and the changes compared with those in the first or control gland.

4. Administration of corticotropin caused an alteration in cytology, in the lipid and cholesterol content of the glands, and in the ribonucleic acid and enzyme content of the cells. The changes resembled those found in the adrenal gland in conditions of stress, and support the view that the adrenal changes in stress are the result of endogenous corticotropin production.

5. The role of adrenal lipids, ribonucleic acid and dehydrogenase enzyme systems in steroid synthesis is discussed.

Acknowledgments

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RENAL RESPONSE TO AMMONIUM CHLORIDE IN GLYCOSURIC OSTEOMALACIA*

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RENAL tubular disorders associated with malacic bone disease have been considered under two major categories. The first, which bears numerous descriptive titles, is thought to result from impaired acid excretion and ammonia synthesis (1). Patients with this disorder (renal hyperchloremic acidosis) manifest an alkaline or slightly acid urine, low serum bicarbonate level, and hyperchloremia. The renal defect, believed to involve the distal portion of the tubule, results in loss of metallic cation, and increased excretion of calcium has been considered particularly significant in the pathogenesis of nephrocalcinosis and bone disease. The second type of tubular abnormality (Fanconi or Detoni-Fanconi syndrome) is thought to involve the proximal convoluted tubule, with resultant glycosuria and increased excretion of amino acids, organic acids and phosphorus. The cause of the bone disease in this variety of tubular insufficiency is not clearly established, but may be the result of urinary loss of phosphorus (2, 3).

Several patients with diagnostic features of the Fanconi syndrome have shown acidosis but its etiology has been considered distinct from that of the acid-base disturbance of renal hyperchloremic acidosis (4). Data presented in several recent reports (5–9) suggest that renal impairment of base conservation may be an associated defect in many instances of the Fanconi syndrome. There is need for more definitive information on this subject, for the traditional explanation that the Fanconi syndrome involves a call on fixed cation because of an increase in organic acid excretion is at variance with many published data.

The present study concerns the blood and urinary changes which accompanied acidosis induced by ammonium chloride in 3 patients with different varieties of glycosuric osteomalacia. The complete data on these patients are to be presented in a separate communication and will be summarized briefly here.

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CASE SUMMARIES

Case I. **E.W.** was a 6-year-old white girl who typified the infantile form of the disorder associated with cystinosis. Rickets, accompanied by glycosuria and acidosis, was first noted during the second year of life. Treatment with large amounts of vitamin D, alkali and phosphate had been applied intermittently but rachitic changes persisted and growth had been markedly retarded. At the time of the present study the patient showed malnutrition, stunting of growth, and the deformities of moderately severe rickets. Cystine crystals were demonstrated by examination of the eyes (slit-lamp) and bone marrow. The urine contained glucose and increased amounts of amino acids. There was marked impairment of endogenous creatinine clearance and elevation of the blood urea nitrogen level.

Case II. **E.C.** was a 16-year-old colored girl who showed multiple tubular reabsorptive defects without cystinosis. Rickets, which failed to heal after administration of the usual antirachitic doses of vitamin D, was first noted at age 2, and all tested urines from that time on contained glucose. At the time of the present study she was of very short stature and exhibited the clinical and radiologic features of rickets and osteomalacia. The major reabsorptive defects of the Fanconi syndrome were present, as well as the chemical features of renal hyperchloremic acidosis. There was neither elevation of blood urea nitrogen level nor impairment of endogenous creatinine clearance.

Case III. **C.C.** was a 45-year-old white man in whom clinical symptoms of osteomalacia developed at the age of 36, and in whom typical laboratory and radiologic features were found one year later. Studies designed to determine the mechanism of the bone disease in this subject have been reported (3). He had glycosuria, moderate and inconstant aminoaciduria, and very marked impairment of phosphate reabsorption. There was no azotemia and results of renal functions tests, including inulin clearance, were within normal limits.

The pertinent laboratory features of these 3 patients are summarized in Table 1, all values representing the average of several determinations.

METHODS

The patients were placed under controlled conditions on the metabolic ward, and a constant diet was started which was calculated to be approximately neutral in ash. Urines were collected for 24-hour periods, put beneath mineral oil after each voiding, stored under refrigeration, and analyzed immediately following termination of each collection period. In Case I shorter periods of collection were made by catheter directly under mineral oil, particularly for measurement of bicarbonate. In the female patients an indwelling catheter was kept in the bladder; the male patient was encouraged to empty his bladder as completely as possible. After a control period, ammonium chloride was given, either as liquid or in the form of non-enteric coated tablets. A Beckman pH meter was used for determination of pH in urine and blood, a closed chamber technique being utilized for the blood samples. Urinary titratable acidity was measured before and after the addition of 0.1 normal hydrochloric acid to the urine specimen, titrating to the blood pH of the patient on that date; it is expressed as "titratable acidity minus CO_2." Urinary ammonium was measured by the method of Folin and Bell (10), and organic acids by that of Van Slyke and Palmer (11). Values for bicarbonate in blood and urine were obtained by the manometric methods of Van Slyke and Neill (12); measurements were

TABLE 1. LABORATORY DATA

	Case 1 (E.W.)	Case 2 (E.C.)	Case 3 (C.C.)
Age	6 yrs.	16 yrs.	45 yrs.
Sex	F	F	M
X-ray diagnosis	Rickets	Rickets and osteomalacia	Osteomalacia
Cystinosis	Yes	No	No
Blood			
Calcium (mg./100 ml.)	9.7	9.5	10.2
Phosphorus (mg./100 ml.)	4.4	2.1	1.8
Alk. p-tase (B.U.)	29.0	48.0	13.0
Sodium (mEq./L)	136.0	137.0	141.0
Potassium (mEq./L)	3.0	3.2	4.5
Chloride (mEq./L)	99.0	115.0	104.0
Bicarb. (mEq./L)	17.0	21.0	28.0
Urea N (mg./100 ml.)	35.0	14.0	17.0
Urine			
Glucose (Gm./day)	++	42.0	15.0
Amino acids (mg./day)	—	1,100.0	400.0
Amino acids (chromatogram)	++++	++++	+
Organic acid (mEq./Kg./day)	9.0	7.5	1.5
Calcium (mg./day)	20.0	240.0	120.0
C* phosphorus uncorrected)	4.5	19.0	34.0
C creatinine (ml./min./1.73 M^2)	30.0	115.0	120.0
C inulin (ml./min./1.73 M^2)	—	—	113.0

* Clearance.

made on "arterialized" venous blood immediately after withdrawal under oil. Chloride was determined by the method of Schales and Schales (13). Sodium and potassium in blood and urine were measured by an internal standard flame photometer, calcium and phosphorus by the respective methods of Clark and Collip (14) and Fiske and SubbaRow (15), and creatinine by the method of Clark and Thompson (16). Urinary amino acids were studied by both paper chromatography and the chemical technique of Albanese and Irby (17).

RESULTS

The findings before and after administration of ammonium chloride in Case I (E.W., the child with cystinosis) are shown in Figure 1. Prior to acid-loading, the serum bicarbonate level was depressed and, although there

was no hyperchloremia, bicarbonate was excreted in the urine.[1] Blood pH was normal but there was slight depression of the serum sodium level and significant hypokaliemia. Endogenous creatinine clearance was less than 30 cc./minute (corrected) and blood urea nitrogen concentration was 33 mg./100 ml. During the administration of 40 to 60 mEq. of ammonium chloride, urinary titratable acidity increased significantly but ammonium

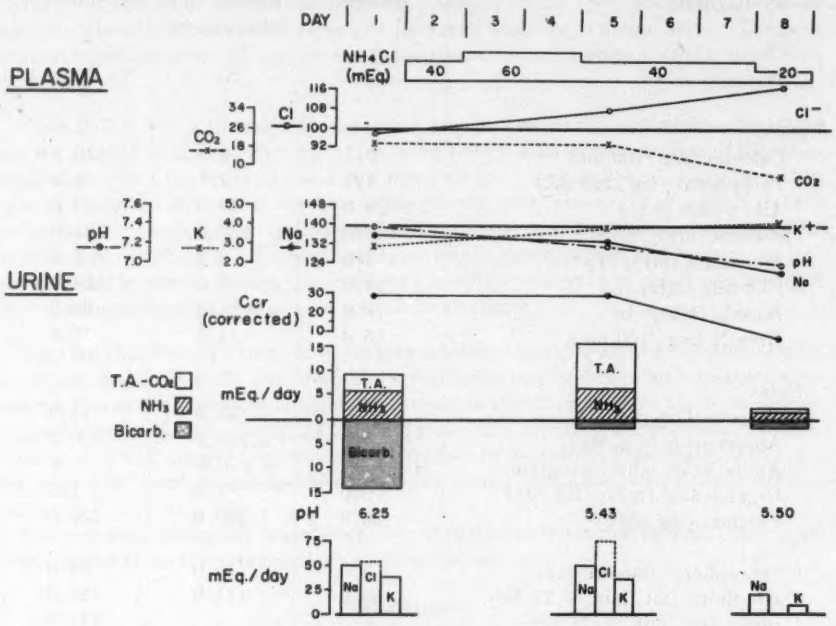

FIG. 1. Findings before and after administration of ammonium chloride in Case I.

excretion did not rise. With continued acid-loading, hyperchloremia became prominent, the serum bicarbonate level was further depressed, serum sodium concentration diminished and there was a fall in blood pH. Six days of acidification resulted in more severe systemic acidosis, decrease of the already impaired creatinine clearance, and severe oliguria. During acidification there was no increase in excretion of sodium or potassium, and chloride excretion failed to keep pace with the administered dose.

Figure 2 depicts the changes in Case II (E.C.). This girl showed evidence

[1] It is apparent that excretion of the amount of bicarbonate shown in Figure 1 is incompatible with the expressed urinary pH (6.25). In view of the fact that urinary pH in this patient was usually between 6.5 and 7.0, the measurement was most probably in error on the control day of study.

of tubular impairment without cystinosis. She had hyperchloremia and a low serum bicarbonate level, and excreted large amounts of bicarbonate in the urine. The serum potassium concentration was diminished but blood pH was within the normal range. The administration of 130 mEq. of

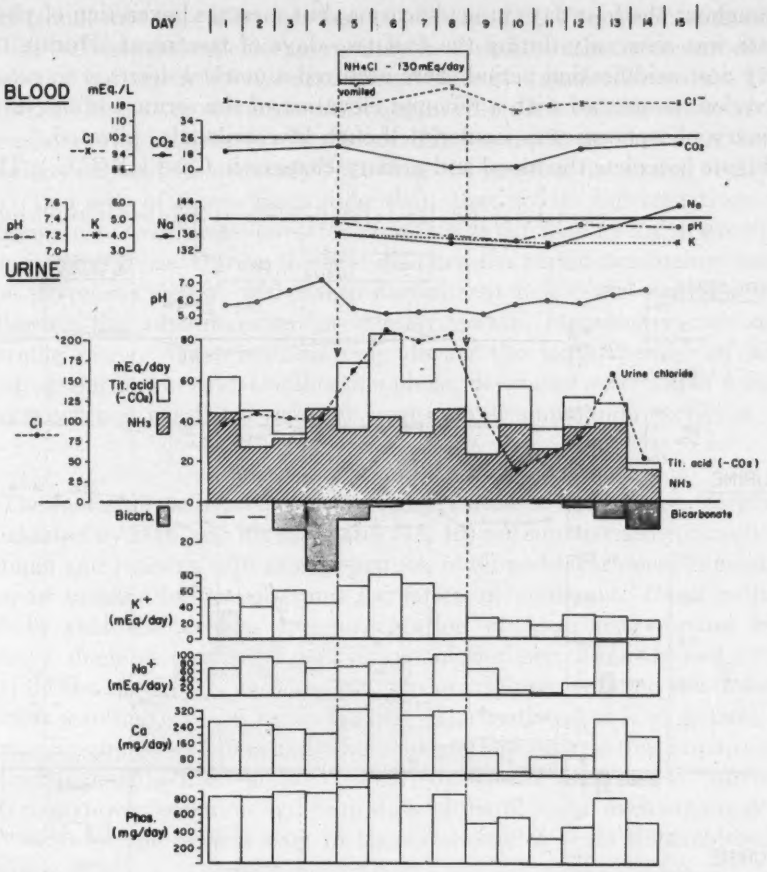

FIG. 2. Findings before and after administration of ammonium chloride in Case II.

ammonium chloride each day for four days resulted in a moderate increase in urinary titratable acidity but ammonium excretion did not rise. Consequently there was a further increase in the concentration of serum chloride and a more marked depression in that of serum bicarbonate. As the latter fell to levels of 10–12 mEq./L, bicarbonate disappeared from the urine in a stepwise manner, reappearing in similar fashion as the serum

bicarbonate concentration returned toward control levels during the post-acidification period. During administration of ammonium chloride the serum sodium level became depressed and blood pH decreased. Chloride excretion lagged slightly behind the amount ingested, particularly on the first day of acidification. Increased excretion of cation was prominent throughout the four days of acidification, but increased excretion of phosphate was seen only during the first two days of treatment. During the early post-acidification period there occurred a marked decrease in cation excretion, associated with a rebound elevation of the serum sodium level. Urinary phosphorus was similarly, though less strikingly, affected.

Figure 3 depicts the blood and urinary changes in Case III (C.C.). This

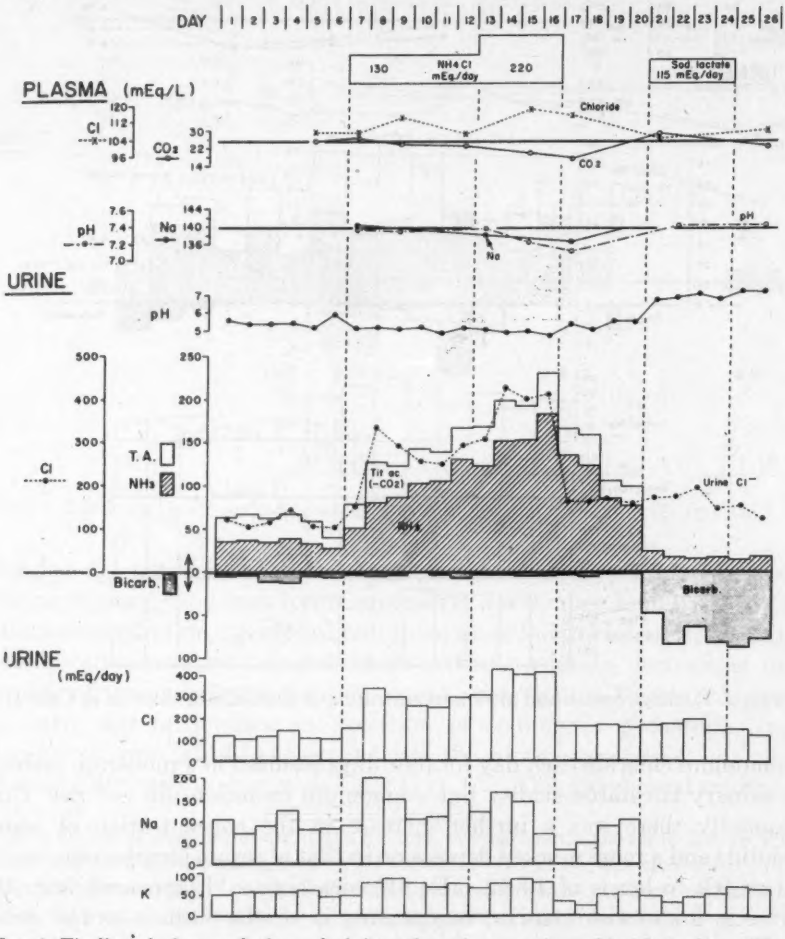

FIG. 3. Findings before and after administration of ammonium chloride in Case III.

man had severe osteomalacia, glycosuria and hyperphosphaturia. The serum sodium, chloride and bicarbonate concentrations were normal, as was the blood pH, and only small amounts of bicarbonate were excreted in the urine. The administration of 130 mEq. of ammonium chloride caused increased excretion of titratable acids and ammonium, the latter attaining a maximal rate only after six days of acidification. The administration of additional ammonium chloride resulted in a further increase in ammonium excretion but, again, several days were required for maximal response. Consequently there was elevation of the serum chloride level, depression of the bicarbonate and sodium levels, and a fall in blood pH. On the initial day of acidification and immediately following the increase in dosage, chloride excretion was of lesser magnitude than that to be expected from the administered load, but thereafter excretion of the loading anion exceeded the amount given. During the post-acidification period ammonium excretion decreased slowly, with sharp curtailment below the control values following the administration of sodium lactate. Increased excretion of metallic cation was prominent only during the initial periods of acid-loading; during the post-acidification phase, decreased excretion of sodium and potassium paralleled the slow regression of ammonium excretion.

DISCUSSION

The mechanisms involved in the conservation of fixed cation, carefully elucidated by Pitts and his associates (18, 19) are summarized concisely by Gilman and Brazeau (20) as reabsorption of filtered bicarbonate, acidification of urinary buffer salts and formation of ammonia. These authors believe that the devices for conservation of bicarbonate-bound base share a single mechanism. Observations of Berliner, Kennedy and Orloff (21) on the effects of a carbonic anhydrase inhibitor indicate this mechanism is secretion of H-ion by the tubular cells, mediated, at least in part, by carbonic anhydrase. These and other observations suggest that impairment of carbonic anhydrase activity, with consequent decrease in proximal bicarbonate reabsorption and limitation of distal H-ion exchange may be the cause of the defects seen in the syndrome of renal hyperchloremic acidosis (22).

Albright and his associates first demonstrated that patients with the syndrome of renal hyperchloremic acidosis lack the capacity to increase the excretion of titratable acids and ammonium, and therefore fail to compensate adequately for acidification with ammonium chloride (23). Consistent changes follow ammonium chloride loading in the normal subject (24, 25). There is an increase in titratable acids and, more strikingly, of ammonium—the latter attaining a maximal rate of excretion only after several days of stimulation. Prior to complete compensation there occurs an increase in urinary excretion of metallic cation. During the post-

acidification period, ammonium excretion slowly declines, and at this time it appears that the organism is able to replenish its stores of fixed cation. Numerous attempts to define the exact stimulus necessary for ammonium excretion have been inconclusive.

Impairment of the mechanisms for conservation of cation is not unique to the syndrome of renal hyperchloremic acidosis but is also a feature of chronic renal disease. Van Slyke and his associates (26) observed that the administration of ammonium chloride to patients with chronic nephritis stimulated excretion of titratable acids without material enhancement of ammonium excretion. Linder (27) demonstrated a similar impairment of ammonium excretion in response to acidification with hydrochloric acid in patients with chronic nephritis. Although deficient ammonium excretion in such cases might result from impaired H-ion secretion, with consequent limitation of ammonia transport, it appears entirely credible that defective ammonia production, possibly the result of inadequate amounts of substrate or impaired enzyme activity, could result in limitation of ammonium excretion even under conditions of normal ion exchange.[2]

Although several patients with glycosuric osteomalacia have demonstrated systemic acidosis and low serum bicarbonate concentration the Fanconi syndrome has been considered entirely distinct from renal hyperchloremic acidosis. Recent reports indicate the need for reconsideration of this viewpoint. Milne, Stanbury and Thomson (6) studied an adult patient with the reabsorptive defects of the Fanconi syndrome who also showed hyperchloremia and impaired bicarbonate reabsorption. His low rate of ammonium excretion was considered appropriate in relation to the urinary pH but supranormal considering the estimated mass of surviving tubular tissue. Precise evaluation of the degree of tubular impairment was complicated by the existence of severe glomerular insufficiency. Sirota and Hamerman (7) recently described an adult with osteomalacia and the tubular reabsorptive defects of the Fanconi syndrome who also demonstrated the typical features of renal hyperchloremic acidosis. The response of urinary titratable acids and ammonium during three days of ammonium chloride stimulation was markedly deficient, even when corrected for loss of renal mass. However, this patient had generalized renal insufficiency with a markedly impaired filtration rate, possibly attributable to associated myeloma. Furthermore, a longer period of acidification may be required to effect peak efficiency of the mechanisms which conserve sodium. The 2 patients reported by Salassa and his associates (8) had, in addition to

[2] The terminology used here is in accord with the theory that NH_3, produced in the tubular cell, continues to diffuse into the tubular lumen only if there exists adequate H-ion to permit formation of NH_4. It is not meant to imply that an alternative hypothesis *i.e.*, production of NH_4 within the cell, may not be correct.

the reabsorptive abnormalities of the Fanconi syndrome, the features of hyperchloremic acidosis. The administration of ammonium chloride, which was poorly tolerated in both instances, was said to have caused increased ammonium excretion but the reported data indicate that the combined increase in titratable acids and ammonium was not proportional to the acid load. The patient with abnormalities of the Fanconi syndrome reported by Linder, Bull and Grayce (5) was not acidotic, but a very inadequate rise in excretion of titratable acids and ammonium resulted from the administration of hydrochloric acid. The adult patient reported by Dragsted and Hjorth (9) showed not only the reabsorptive defects of the Fanconi syndrome but also hyperchloremia and a depressed serum bicarbonate level. The administration of ammonium chloride caused a significant fall in urinary pH, but no data dealing with ammonium excretion were presented. The 2 patients with associated skeletal and tubular disease reported by Hiltemann and co-workers (28) had glycosuria and a low serum bicarbonate level without hyperchloremia; ammonium excretion was not increased. It appears from these studies that some of the patients with tubular reabsorptive defects associated with the Fanconi syndrome also demonstrate features consistent with the syndrome of renal hyperchloremic acidosis. A complete evaluation of the range of defects in published cases is made difficult by unduly rigid classification, lack of uniform methods of study, and the usual task of determining whether impaired functions are specific to the Fanconi syndrome or secondary to pyelonephritic or diffuse renal damage. It is believed that the studies reported here may help to clarify the problem.

E.W. (Case I) is considered representative of the infantile form of glycosuric rickets with cystinosis, recently classified by Bickel and his associates (29) as "Lignac-Fanconi Disease." Despite the presence of acidosis and increased urinary bicarbonate excretion there was no hyperchloremia. Although the inadequate response to acidification was consistent with the changes seen in the syndrome of renal hyperchloremic acidosis, the lack of hyperchloremia, the marked impairment of glomerular filtration and some features of the response to acidification (namely, rise in titratable acidity without increase in ammonium excretion) are more in accord with the response to acidification noted in chronic nephritis. It would appear that at least part of the abnormalities of cystinosis are due, not to an inborn error of tubular function, but rather to secondary renal damage induced by the cystine deposits. These views are in accord with those of Bickel and associates (29), who have amassed convincing evidence that many of the abnormalities in cystinosis are secondary to malnutrition and renal damage. However, the microdissection studies of Clay, Darmady and Hawkins (30) revealed significant morphologic abnormalities in 2

patients with the Fanconi syndrome, 1 of whom had cystinosis. The most characteristic abnormality—narrowing of the upper portion of the proximal convoluted tubule—did not appear to be an acquired change.

E.C. (Case II) revealed all of the tubular reabsorptive defects of the Fanconi syndrome but demonstrated neither cystinosis nor impairment of glomerular filtration. The hyperchloremia, low serum bicarbonate concentration, and excessive bicarbonate excretion were entirely consistent with the changes seen in renal hyperchloremic acidosis. Her response to acidification was grossly inadequate, particularly as concerned ammonium excretion. Certain features of the response were of special interest. When the serum bicarbonate level was moderately depressed, large amounts of bicarbonate were excreted in the urine; but during acidification, when the serum bicarbonate level was further decreased, bicarbonate reabsorption appeared to have become complete (Fig. 2). Latner and Burnard (31) have advanced the idea that in the syndrome of renal hyperchloremic acidosis there is no limitation of H-ion secretion but, rather, competition for available H-ion in the distal tubule because of impaired proximal tubular bicarbonate reabsorption. This concept implies that either deficient stimulation of ammonia formation or insufficient H-ion to permit its continued transport into the tubular lumen results in the low rate of ammonium excretion. Note that in Case II (Fig. 2), even after bicarbonate reabsorption had apparently become complete, with a marked fall in urinary pH, ammonium excretion still failed to rise. The concept of Latner and Burnard was based on a rise in titratable acidity and ammonium, together with an increase in urinary pCO_2 to levels exceeding those in plasma, as observed during phosphate infusion in cases of hyperchloremic acidosis. Studies by Kennedy, Orloff and Berliner (32) reveal the inadequacy of urinary pCO_2 as a significant measurement of distal tubular integrity. Milne *et al.* (6) and Smith and Schreiner (22) found that infusion of phosphate in patients with renal hyperchloremic acidosis failed to elicit the rise in ammonium excretion described by Latner and Burnard. The response to acidification reported in the present study offers further evidence that the impaired ammonium excretion is not attributable to flooding of the distal tubule with bicarbonate.

Despite impairment of ammonium excretion, there was a significant rise in "titratable acidity minus CO_2" which compared favorably with that seen in patients with apparently normal renal tubular function. The studies of Albright and associates demonstrated a similar rise in titratable acidity in patients with renal hyperchloremic acidosis, the major abnormality being an inadequate rise in ammonium excretion in answer to the challenge of acidosis (23). It seems possible that recent preoccupation with H-ion secretion may have diverted attention from earlier concepts regarding a

possible defect in renal hyperchloremic acidosis, namely, specific limitation of ammonia formation.

Another point of interest in Case II was the marked decrease in excretion of potassium, sodium and calcium, associated with a rebound elevation of the serum sodium level, which followed cessation of stimulation by ammonium chloride. These features, observed in normal subjects during the post-acidification period, have been attributed to slow regression of ammonia formation (33). Insomuch as this patient (Fig. 2) failed to demonstrate any *increase* in ammonium excretion, either during or following acid loading, restoration of fixed cation must be attributed to some other mechanism.

C.C. (Case III), although demonstrating both glycosuria and severe osteomalacia, was not acidotic. Ammonium chloride stimulation resulted in a normal increase in urinary base-sparers, ammonium excretion rising slowly, as is the rule in normal subjects. A further increment of acid, after compensation to the initial load had been attained, resulted in a similar slow increase in urinary ammonium. Excretion of loading anion and increased excretion of metallic cation followed a pattern harmonious with that which occurs in subjects with normal faculties for acidification of the urine. Studies in this patient demonstrated the presence of normal urinary acidification with clear implication that urinary cation loss secondary to acidosis could not be advanced in explanation of the demineralization of bones.

The 3 patients discussed in this report appear representative of the types of tubular abnormality which have been grouped, perhaps too broadly, under the term Fanconi syndrome. Their study indicates that entirely dissimilar disorders may share certain common features, such as osteomalacia, glycosuria and hyperphosphaturia. Observations in Case I suggest that the renal deficiency in conservation of base which is observed in cystinosis is secondary to diffuse renal damage, rather than to an intrinsic tubular defect. The findings in Case III, which are consistent with those of several reported instances of adult glycosuric osteomalacia, indicate that this bone disease has an entirely different etiology from that of "tubular rickets" associated with renal hyperchloremic acidosis. Case II represents a blend of the two traditionally dissimilar disorders, *i.e.*, renal hyperchloremic acidosis and the Fanconi syndrome. The abnormalities seen in this patient, as well as similar findings in several other reported instances of glycosuric osteomalacia, suggest that these two tubular disorders may be very closely related. Despite the fact that several patients mentioned in this report had, in addition to the features of the Fanconi syndrome, defects in conservation of bicarbonate-bound base, no particular note has been made of the existence of defective tubular reabsorption in patients with the classic features

of renal hyperchloremic acidosis. The fact that 1 of Albright's original patients with "tubular insufficiency without glomerular insufficiency" (M.St.P., MGH #237547) was noted to have glycosuria (34), the subsequent observation that this patient and 1 other with the same syndrome excreted abnormal amounts of organic acids (35), and the recent implication of renal hyperchloremic acidosis as a familial disorder (36), offer further evidence for a close association between the two syndromes.

SUMMARY

Three patients with different varieties of glycosuric rickets were studied with respect to their renal response to acid loading. Two of the patients, both of whom had acidosis prior to treatment, demonstrated gross defects of conservation of bicarbonate-bound base; the third patient had no such defect. The changes observed in these patients permit the following suggestions:

1. Certain features of the response to acid loading in cystinosis appear to be more characteristic of chronic renal disease than of pure limitation of distal tubular function.

2. Specific impairment of ammonia formation may be a major defect in some instances of renal hyperchloremic acidosis, at least when complicated by the presence of reabsorptive abnormalities considered characteristic of the Fanconi syndrome.

3. Factors other than cation loss resulting from renal acidosis must be implicated as the cause of bone disease in some instances of glycosuric osteomalacia.

4. The two renal tubular syndromes, renal hyperchloremic acidosis and the Fanconi syndrome, are not always clearly separable and may be closely related.

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SPECIES VARIATIONS IN THE RESPONSE OF SERUM PROTEINS, LIPIDS AND LIPOPROTEINS TO THE CORTISONES*†

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PREVIOUS investigations have established the normal lipid partition and lipoprotein profile in man, dog and rabbit (1, 2). Elevation of serum lipid fractions has been reported in patients treated with prolonged and large dosages of cortisone (3–5), and in rabbits and dogs receiving cortisone acetate or hydrocortisone acetate (6, 7). Hypercholesteremia has been observed in patients with Cushing's syndrome (8). Alteration of plasma protein pattern has been noted in dogs receiving cortisone (9). A correlative study of the influence of cortisone and other corticosteroids on the levels of serum and plasma proteins, lipids and lipoproteins in various species was therefore undertaken.

MATERIALS AND METHODS

The levels of proteins and lipoproteins in serum or plasma were determined simultaneously by means of paper electrophoresis. A horizontal apparatus with free suspension of the paper strips was employed. The buffer was veronal (pH 8.6, ionic strength 0.05). For protein determinations, .005 cc. of serum was streaked across filter paper strips 2.5 cm. wide (Whatman 3 MM); .03 cc. was applied to parallel strips for lipid determinations. A current of 12–15 milliamperes (potential difference, 250 volts) was applied for seven hours with six strips in place. After drying at 70° C., parallel strips were stained with Amidoschwarz 10B for proteins and Oil Red O for lipids. Densitometric evaluation was performed with a Photovolt Model 525 instrument. The resultant curves were analyzed by planimetry. Chemical estimation of the concentration of individual lipid fractions was performed on the same samples. Free and esterified cholesterol, phospholipid and total lipid levels were determined by the methods of Schoenheimer and Sperry, Fiske and SubbaRow, and Bloor, respectively (10–12). Neutral fat was calculated by subtracting the value for total cholesterol plus phospholipids from the value for total lipid.

The control group of human beings included normal men and women, aged 18 to 45

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years, clinically free of disease and examined after a fast of twelve to fourteen hours. Three female patients, aged 28, 38, and 44 years, who were being treated with large doses of cortisone for pulmonary interstitial fibrosis and/or hemolytic anemia in the course of Hodgkin's disease were investigated; they received an average daily dose of 200 mg. of cortisone for five weeks, one year, and seven weeks, respectively. Studies were also made in 2 women with Cushing's syndrome attributed to adrenal hyperplasia; they were 23 and 34 years old and had had symptoms of their disease for four years and for six months, respectively. In both patients the urinary 11-oxygenated steroid excretion was considerably elevated (6.0 mg. and 3.8 mg. in twenty-four hours), but the 17-ketosteroid excretion only slightly increased (23.1 mg. and 18.5 mg. in twenty-four hours). Serum lipid partition was studied serially in 22 patients (7 males and 15 females; average age, 37 years) who were receiving cortisone acetate orally for treatment of a variety of diseases, including disseminated lupus erythematosus, rheumatoid arthritis and leukemia. The duration of treatment varied from six to one hundred and five days, averaging twenty-five days, and the total dose of steroid used ranged from 0.5 to 7.2 Gm. (average dose, 2.18 Gm.).

Heparinized plasma was examined from normal male mongrel dogs weighing 11 to 22 Kg., maintained on a diet of meat, vegetables and milk and fasted for sixteen hours. Dogs were studied after daily injections of 100 or 200 mg. of cortisone acetate over a period of one to four weeks. Additional investigations were carried out after parenteral administration of 100 mg. of hydrocortisone acetate for three weeks and after injection of a similar dose of 1-dihydrocortisone (prednisone) for ten days.

Heparinized plasma was examined from fasted normal male rabbits, including albino, chinchilla and "giant checker" strains, weighing 2 to 3 Kg. The experimental group included rabbits receiving daily injections of cortisone acetate, 1.25 to 5 mg. per Kg. for from ten to sixty days; hydrocortisone acetate, 1.25 to 2.5 mg. per Kg for from ten to thirty-three days, or 1-dihydrocortisone, 1.2 mg. per Kg. for from ten to thirty-three days.

RESULTS

The protein patterns were analyzed into albumin, alpha-1, alpha-2, beta and gamma globulins—and fibrinogen when plasma was studied. In the rabbit, the marked variability in the number of alpha components precluded separation within this group. Albumin levels were highest in man, lowest in the dog and intermediate in rabbits. In all instances, the gamma fraction was the predominant globulin component. In the dog there was relatively more beta globulin, as compared to the other species.

Lipoproteins were divided into alpha-, beta-, and O-fraction (lipid adsorbed at the origin). The lipoprotein profile was similar in man and rabbit, except for relatively larger amounts of the O-fraction in the latter. The lipoprotein profile of the dog was almost the reverse of that in the other species.

Man was characterized by the highest levels of serum total and esterified cholesterol, and the dog by the highest levels of phospholipids, neutral fat and total lipid. Rabbits exhibited low levels of all lipid fractions, particularly cholesterol and total lipid (Table 1).

TABLE 1. SERUM (PLASMA) PROTEIN PATTERN, LIPOPROTEIN PROFILE AND LIPID PARTITION IN VARIOUS SPECIES

Species	No. of subjects	*Proteins*				
		Albumin	Globulins			
			Alpha-1	Alpha-2	Beta	Gamma (plus fibrinogen)
		(% of total stainable protein)				
Man	12	52.5	4.2	12.2	14.0	17.1
Dog	6	38.8	4.6	9.1	21.1	26.4
Rabbit	5	47.0	14.1		17.0	21.9
		Lipoproteins				
		Alpha	Beta		O	Beta plus O
		(% of total stainable lipid)				
Man	10	35.3	52.4		12.3	64.7
Dog	8	69.3	22.1		8.6	30.7
Rabbit	16	30.7	50.4		18.9	69.3
		Lipids				
		Cholesterol/ esters	Phospho-lipids		Neutral fat	Total lipid
		(mg. per 100 ml.)				
Man	119	211/157	242		176	629
Dog	23	158/120	303		266	727
Rabbit	119	49/ 37	103		198	350

The patients under long-term therapy with large doses of cortisone all had hypoalbuminemia, which may have been related to their underlying disease. Relative increases in gamma and beta globulins were noted. The patients with Cushing's syndrome had a lesser degree of hypoalbuminemia and less marked, but similar trends in elevation of the globulin levels. Both groups of patients were characterized by a significant increase in the O-fraction to about one-fourth the total stainable lipid (Table 2).

Serial study of serum lipids in the patients under cortisone therapy revealed in most instances an elevation of total and esterified cholesterol

TABLE 2. EFFECT OF CORTISONE ON SERUM PROTEIN PATTERN AND LIPOPROTEIN PROFILE IN MAN

Group	No. of subjects	*Proteins*				
		Albumin	Globulins			
			Alpha-1	Alpha-2	Beta	Gamma
		(% of total stainable protein)				
Control	12	52.5	4.2	12.2	14.0	17.1
Cortisone therapy	3	31.5	6.6	12.7	18.1	31.1
Cushing's syndrome	2	39.8	9.2	13.2	18.3	19.4
		Lipoproteins				
			Alpha	Beta	O	Beta plus O
		(% of total stainable lipid)				
Control	10		35.3	52.4	12.3	64.7
Cortisone therapy	3		29.0	40.4	30.6	71.0
Cushing's syndrome	2		37.5	37.7	24.8	62.5

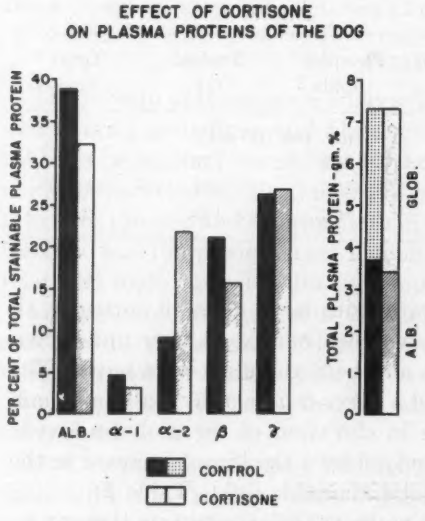

FIG. 1. Plasma protein pattern of normal dogs compared to that in dogs receiving cortisone acetate.

levels, amounting to an average increase of 20 per cent. Hypercholesteremia was observed in 35 per cent of the subjects and developed three times more frequently in patients whose courses of therapy were prolonged beyond sixty days. Hyperphospholipidemia regularly accompanied the hypercholesteremia. In some instances the serum became turbid (4, 5).

A selective elevation of alpha-2 globulin amounting to double the normal value was noted in the plasma of dogs receiving cortisone (Fig. 1). There was an associated drop in beta globulin. The shift in plasma globulin distribution occurred in the absence of hypoalbuminemia or change in total protein (9). Similar

changes were observed in dogs treated with 1-dihydrocortisone. No significant alteration was noted in the plasma lipoprotein profile or lipid partition in dogs injected with cortisone or hydrocortisone within the experimental period (Table 3).

Contrariwise, in rabbits no alteration was observed in plasma protein pattern after administration of cortisone, hydrocortisone or 1-dihydrocortisone (Table 4). There was marked alteration in the lipoprotein profile, however, with marked depression of alpha-lipoprotein, a moderate

TABLE 3. EFFECT OF CORTICOSTEROIDS ON PLASMA LIPID PARTITION AND LIPOPROTEIN PROFILE IN DOGS

Group	No. of subjects	*Lipids*			
		Cholesterol/ esters	Phospho- lipids	Neutral fat	Total lipid
		(mg. per 100 ml.)			
Control	23	158/120	303	266	727
Cortisone or Hydrocortisone	7	171/130	310	252	733
		Lipoproteins			
		Alpha	Beta	O	Beta plus O
		(% of total stainable lipid)			
Control	8	69.2	22.1	8.6	30.7
Cortisone	2	72.2	18.5	9.3	27.8

drop in beta-lipoprotein and marked elevation of the O-fraction. Such changes were most pronounced following administration of 1-dihydrocortisone, whereas hydrocortisone had the least effect. These animals also showed a significant elevation of all lipid fractions, with the most marked increase in total lipid and neutral fat to four or five times the control levels. The plasma became lactescent. Total cholesterol and phospholipid levels increased two- to threefold. Similar levels of lipid fractions were observed following administration of cortisone and 1-dihydrocortisone, and lower elevations were observed in rabbits receiving hydrocortisone. Administration of 1-dihydrocortisone resulted in a marked depression of the ratio of esterified to total cholesterol (Table 4). The effect of administration of 1-dihydrocortisone (prednisone) on the level of plasma lipids in a rabbit followed serially is depicted in Figure 2.

TABLE 4. EFFECT OF CORTICOSTEROIDS ON PLASMA PROTEIN PATTERN, LIPID PARTITION AND LIPOPROTEIN PROFILE IN RABBITS

Group	No. of subjects	*Proteins*				
		Albumin	Globulins			
			Alpha	Beta	Gamma (plus fibrinogen)	
		(% of total stainable protein)				
Control	5	47.0	14.1	17.0	21.9	
Cortisone	2	44.7	17.0	17.9	20.3	
Hydrocortisone	2	50.1	13.3	16.7	19.9	
1-Dihydrocortisone	3	45.6	15.7	18.1	20.6	
All steroids	7	46.6	15.4	17.7	20.3	
		Lipids				
		Cholesterol/esters	Phospholipids	Neutral fat	Total lipid	No. of days admin'd (avge.)
		(mg. per 100 ml.)				
Control	119	49/37	103	198	350	
Cortisone	18	158/89	278	1019	1454	34
Hydrocortisone	8	95/51	207	802	1105	23
1-Dihydrocortisone	5	114/41	297	1094	1505	30
		Lipoproteins				
		Alpha	Beta	O	Beta plus O	
Control	16	30.7	50.4	18.9	69.3	
Cortisone	2	10.2	30.8	59.0	89.8	14
Hydrocortisone	2	11.8	33.9	54.3	88.2	29
1-Dihydrocortisone	5	5.8	34.5	62.7	97.2	30

DISCUSSION

The species examined showed distinctive patterns of proteins, lipids and lipoproteins. They also showed characteristic species differences in their metabolic responses to administration of corticosteroids.

In man, changes in all three foregoing serum components were observed and they seemed to occur independently of each other. Alteration in lipo-

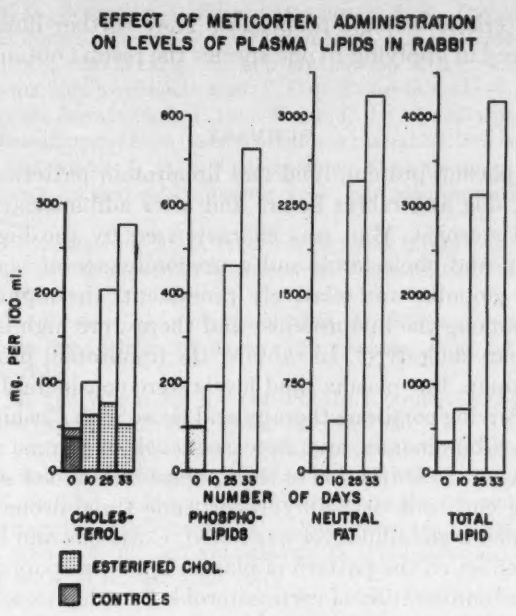

FIG. 2. Plasma lipid partition determined serially in a rabbit receiving daily injections of 1-dihydrocortisone (prednisone; Meticorten), 1.2 mg. per Kg.

protein levels without alteration in serum lipid levels may provide another indication of the effect of the adrenal cortex on lipid metabolism in man.

In dogs, protein metabolism was affected, in that marked alterations in plasma protein patterns were uniformly observed after administration of cortisone and 1-dihydrocortisone. Nevertheless, in this species no alterations in lipoprotein or lipid patterns were noted after injection of cortisone within the experimental period. This may be correlated with the markedly dissimilar lipoprotein distribution in the dog, in which alpha-lipoprotein is the dominant fraction as opposed to the distribution in man and the rabbit.

In rabbits, on the other hand, marked alteration in both plasma lipid and lipoprotein levels were noted while the protein pattern remained unmodified, after administration of corticosteroids. Differences in the effects of the various steroids on lipid metabolism were also observed and merit further investigation. The failure of the esterified cholesterol level to rise after administration of 1-dihydrocortisone suggests the possibility of liver damage in these animals and deserves additional study (13).

The differences in the normal distribution of serum (plasma) proteins, lipids and lipoproteins among the species examined, as well as the varying responses to administration of the cortisones, are further indications of

species-characteristic biologic properties. They further illustrate the pitfalls encountered in applying to one species the results obtained in another species.

SUMMARY

Serum and plasma protein, lipid and lipoprotein patterns were investigated in man, dog and rabbit before and after administration of various adrenocortical steroids. Man was characterized by the highest levels of serum albumin and cholesterol, and a predominance of beta-lipoprotein. In dogs, beta globulin was relatively prominent; the alpha fraction was predominant among the lipoproteins; and there were high levels of serum lipids other than cholesterol. In rabbits, the lipoprotein profile resembled that in the human, but plasma lipid levels were considerably lower.

Humans receiving cortisone therapy and those with Cushing's syndrome exhibited hypoalbuminemia, and increased levels of gamma and beta globulins, as well as of the O-fraction of the lipoproteins, and of serum lipids. In dogs receiving cortisone or 1-dihydrocortisone (prednisone), elevation of the plasma alpha-2 globulin level was noted. Cortisone and hydrocortisone were without effect on the pattern of plasma lipids or lipoproteins. Rabbits responded to administration of corticosteroids by a depression of the alpha-lipoprotein level and marked elevation of the O-fraction, and all plasma lipid fractions were greatly increased, particularly total lipid and neutral fat. The effects observed following administration of 1-dihydrocortisone were more pronounced than those following cortisone and hydrocortisone.

Species characteristics and inter-species differences in the patterns of the serum (plasma) components studied illustrate some of the difficulties involved in correlating metabolic studies in man with those in experimental animals.

Acknowledgments

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THE DIURNAL VARIATION OF PLASMA LEVELS AND URINARY EXCRETION OF 17-HYDROXY-CORTICOSTEROIDS IN NORMAL SUBJECTS, NIGHT WORKERS AND BLIND SUBJECTS*†

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A DIURNAL rhythm in the plasma levels and urinary excretion of corticosteroids by men has been reported previously. Pincus *et al.* (2) and Romanoff *et al.* (3) showed that the rate of urinary excretion of phosphomolybdic acid-reducing lipids and formaldehydogenic lipids was greater during the morning than during the remaining 24-hour period. Using a more specific method of hydrolysis of the urinary corticoid conjugates, Sandberg *et al.* (4) reported the same type of variation; similar results were also reported by Laidlaw *et al.* (5). In a study of the normal plasma levels of 17-hydroxycorticosteroids (17-OH-CS) in man, Bliss *et al.* (6) found that in any single subject, the 8 A.M. values were higher than those found later in the day. We have extended this study to include the entire twenty-four hours, with special emphasis on the night period. Observations were also made in individuals whose pattern of activity and sleep was reversed and in blind persons, in an attempt to determine the effect of activity and the visual perception of light on this diurnal pattern.

METHODS

Experimental subjects

Blood samples from control subjects were collected at 8 A.M., 10 P.M., 4 or 5 A.M., 6 A.M. and 8 A.M. the following morning. Most of these individuals awoke between

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6:30 and 7:30 A.M. and none of them was asleep before 10 P.M. Subjects 1 to 4 were awakened with the minimal disturbance possible at 2 A.M. and 5 A.M. and went back to sleep after blood collections. A polyethylene catheter was introduced into the antecubital vein of Subjects 5 to 12 after the 10 P.M. sample was drawn, in order to permit collection of blood during the night while they were asleep. This procedure was followed in an attempt to minimize the possible disturbance caused by repeated awakenings. Three normal subjects collected their urines in approximately four-hour periods for three days in order to determine the individual day-to-day variation of 17-OH-CS excretion. These subjects retired regularly at midnight and awakened at 8 A.M.

To determine the influence of activity, we studied nurses and watchmen who had been working at night for at least six months prior to the time of the experiment. Subjects 16 to 19 collected their urines in approximately four-hour periods during the test.

Completely blind but otherwise normal subjects had blood samples taken at 8 A.M., noon, 8 P.M., midnight, 6 A.M. and 8 A.M. the following morning. In the blind subjects and in some of the night workers, circulating eosinophils and plasma iron levels were measured simultaneously.

Plasma and urinary 17-hydroxycorticosteroid (17-OH-CS) determinations

Heparinized blood samples were centrifuged immediately and the plasma was separated from the cells. Urine was kept in a refrigerator during collection; following collection, plasma and urine samples were kept in a deep freeze.

Plasma levels were determined by the method of Nelson and Samuels (7) as modified by Eik-Nes *et al.* (8), and urinary 17-OH-CS by the method of Glenn and Nelson (9).

Determination of eosinophils and plasma iron

Circulating eosinophils were estimated on oxalated blood by the method of Randolph (10). Plasma iron levels were determined as described by Hamilton *et al.* (11).

TABLE 1. PLASMA 17-OH-CS LEVELS (μG./100 ML.) IN NORMAL CONTROLS

Subjects			Time						
No.	Age (yrs.)	Sex	8 A.M.	10 P.M.	2 A.M.	4 A.M.	5 A.M.	6 A.M.	8 A.M.
1	29	♂	17.8	6.6	7.5	—	10.	—	22.
2	41	♂	15.5	6.5	6.0	—	18.3	—	17.9
3	32	♀	16.4	9.9	4.4	—	12.8	—	19.2
4	38	♀	15.7	8.6	7.4	—	16.1	—	16.9
5	39	♂	20.4	0.	4.8	5.8	—	25.2	14.5
6	53	♂	16.8	6.5	8.6	10.	—	31.2	20.
7	18	♂	15.4	11.2	—	21.6	—	19.	13.6
8	30	♀	11.3	7.9	—	9.6	—	14.	10.7
9	30	♂	14.	0.5	6.2	—	10.8	16.4	12.
10	25	♂	9.	7.3	9.8	—	—	12.8	14.
11	26	♂	4.7	0.8	—	—	3.4	—	5.7
12	46	♂	18.	2.1	1.3	8.4	—	18.9	18.3

TABLE 2. PLASMA 17-OH-CS LEVELS (μG./100 ML.) IN NIGHT WORKERS

Subjects			Time							
No.	Age (yrs.)	Sex	12 M.	4 A.M.	6 A.M.	8 A.M.	12 N	4 P.M.	8 P.M.	10 P.M.
16	22	♂	10.5	13.	18.9	—	12.3	12.3	—	0.3
17	27	♂	14.5	10.5	12.9	—	6.3	2.0	—	0.9
18	21	♂	8.6	8.2	10.3	—	7.5	5.2	—	5.7
19	25	♂	6.1	7.	11.6	—	9.2	4.5	—	5.8
20	22	♂	7.1	7.5	16.3	17.5	—	12.5	5.5	—
21	38	♀	5.9	4.6	10.8	6.8	—	4.1	5.0	—
22	43	♂	1.5	4.3	12.	—	13.3	6.4	3.7	—
23	40	♂	0.	5.9	20.6	20.8	6.4	8.5	0.	—
24	39	♂	—	4.9	17.3	12.4	11.8	2.	2.	—
25	23	♀	3.8	17.5	—	10.3	6.4	9.6	0.	—

RESULTS

The levels of 17-OH-CS in the plasmas of the normal subjects and of the night workers are listed in Tables 1 and 2. The mean values of the normal subjects are presented graphically (Figs. 1 and 2). Figure 2 shows the means of the values for the night workers compared with a larger heterogeneous series of values obtained in normal individuals in our laboratory at various times. The standard errors of the means used to construct the latter curve averaged approximately 1 μg./100 ml.

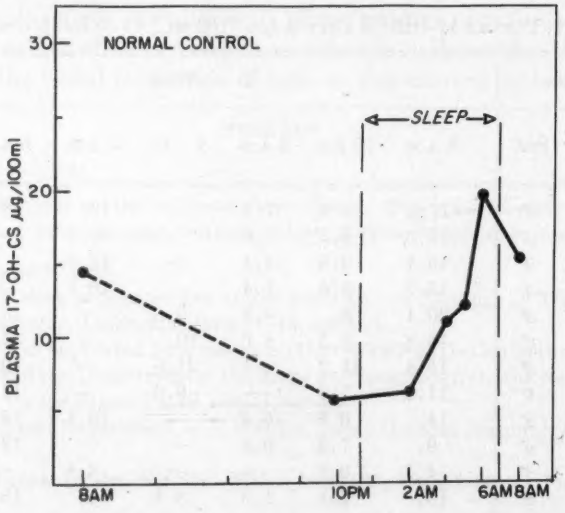

FIG. 1. Mean variation of plasma 17-OH-CS levels in normal subjects during the night.

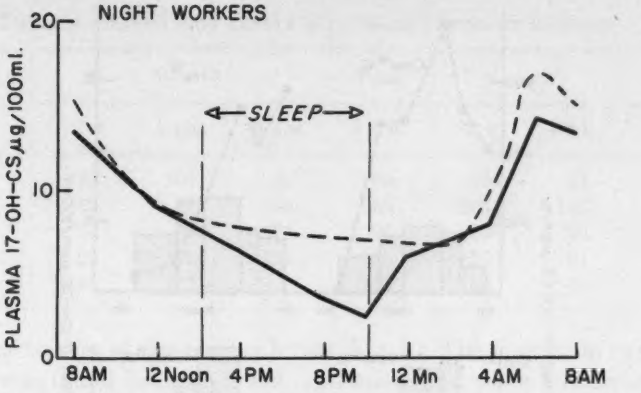

FIG. 2. The mean variation of plasma 17-OH-CS levels in night workers is shown by the heavy line. Their usual period of sleep is also shown. The interrupted line is derived from a very large series of values in normal subjects on a usual schedule of activity and rest, and is presented to show the similarity of the two curves.

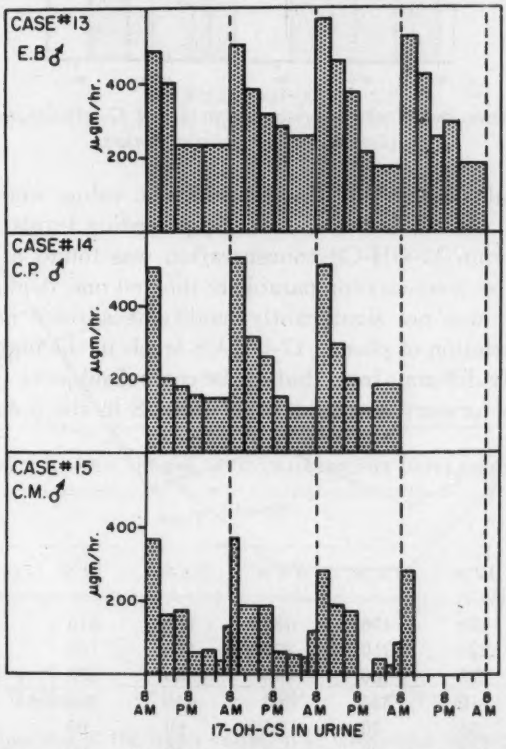

FIG. 3. Diurnal variation in the rate of excretion of urinary 17-OH-CS in 3 normal young adults.

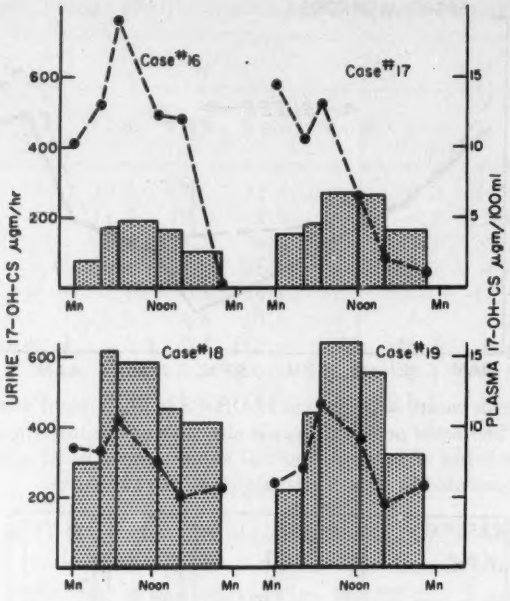

FIG. 4. Variation in the rate of urinary excretion of 17-OH-CS in comparison to plasma levels in 4 night workers.

In the normal subjects the 10 P.M. and 2 A.M. values were the lowest of the day. By 4 A.M. an increase over the preceding levels was apparent, and the maximum 17-OH-CS concentration was found at about 6 A.M. The second 8 A.M. level was comparable to the first one, demonstrating that the experiment was not significantly modifying adrenal hormone levels. The diurnal variation of plasma 17-OH-CS levels in the night workers was not significantly different from that of the control subjects.

The curve of urinary excretion of 17-OH-CS in the 3 control subjects

TABLE 3. CIRCULATING EOSINOPHILS (CELLS PER CU. MM.) IN NIGHT WORKERS

Subject	Time						
	12 M	4 A.M.	6 A.M.	8 A.M.	12 N	4 P.M.	8 P.M.
20	420	450	350	150	310	305	400
21	225	310	275	175	150	155	150
22	225	400	335	350	305	340	345
23	710	355	365	345	385	315	385
24	85	70	95	60	65	65	135
25	100	80	60	40	60	90	135

TABLE 4. PLASMA IRON LEVELS (μG./100 ML.) IN NIGHT WORKERS

Subject	Time						
	12 M	4 A.M.	6 A.M.	8 A.M.	12 N	4 P.M.	8 P.M.
20	133	102	93	90	99	84	113
21	62	65	154	289	203	145	183
22	57	66	74	96	93	99	99
23	97	102	124	165	157	91	60
25	141	117		100	109	86	80

was similar to that of the plasma levels (Fig. 3). The maximum excretion, however, was found between 8 A.M. and noon, and when a collection was made from 8 to 10 A.M. (No. 15) this sample showed the highest rate of 17-OH-CS excretion. Similar patterns were observed in the night workers (Fig. 4). It would appear that there is approximately a two-hour delay between the peak of the plasma level and the highest rate of urinary excretion.

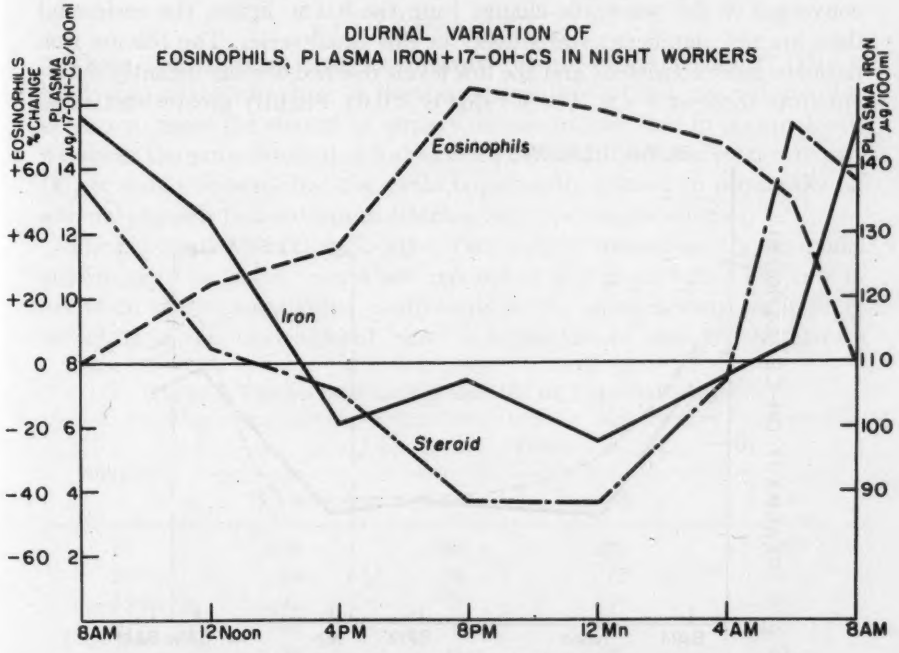

FIG. 5. Comparison of the mean variation of circulating eosinophils, plasma iron and plasma 17-OH-CS levels in the night workers.

TABLE 5. PLASMA 17-OH-CS (μG./100 ML.) IN BLIND SUBJECTS

Subjects			Time					
No.	Age (yrs.)	Sex	8 A.M.	12 N	8 P.M.	12 M.	6 A.M.	8 A.M.
26	35	♂	25.2	9.6	12.0	8.9	15.5	18.2
27	48	♂	14.1	6.1	9.1	8.3	15.4	13.1
28	54	♂	9.0	4.3	7.7	6.6	13.5	14.5
29	25	♀	11.1	6.4	4.8	4.1	12.3	11.6
30	44	♂	9.4	6.0	3.4	6.0	17.2	10.1
31	34	♀	9.9	4.0	4.8	3.1	10.0	9.6

In Tables 3 and 4 the data concerning the eosinophil and plasma iron variations in these night workers are presented. Like the 17-OH-CS levels, the mean curves show a striking pattern (Fig. 4). The plasma iron values have a similar periodicity but the cycle is a few hours later, whereas the eosinophil count shows an inverse curve (Fig. 5). It should be noted, however, that because of the wide variation in individual values, even when converted to the percentile change from the 8 A.M. figure, the eosinophil data are not significant statistically in this small series. The plasma iron data are more consistent and the low levels reached are significantly different from those at 8 A.M. The p value is <0.01. Slightly greater statistical

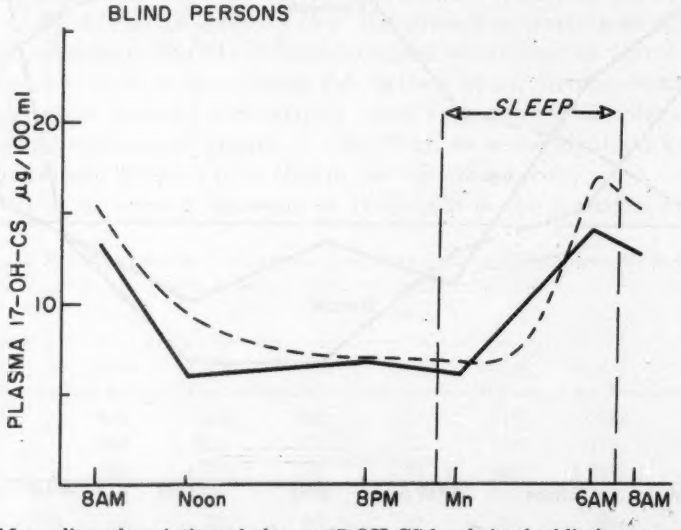

FIG. 6. Mean diurnal variation of plasma 17-OH-CS levels in the blind persons (heavy line) compared to that in a large series of normal subjects (interrupted line).

TABLE 6. CIRCULATING EOSINOPHILS (CELLS PER CU. MM.) IN BLIND SUBJECTS

Subjects	Time					
	8 A.M.	12 N	8 P.M.	12 M.	6 A.M.	8 A.M.
26	220	85	140	260	145	85
27	145	125	120	215	160	155
28	110	155	100	175	160	110
29	410	275	260	395	210	270
30	110	75	140	175	130	125
31	145	110	240	245	110	165

significance is found between the high and low steroid values in this series.

The diurnal variation of 17-OH-CS levels in the plasma of blind persons was similar to that found in normal subjects (Table 5 and Fig. 6). Although the magnitude of the mean variation was somewhat less, the differences are not significant statistically in this small series. The average changes in eosinophils and plasma iron levels, however, were smaller than those in the night workers (Tables 6, 7 and Fig. 7).

DISCUSSION

These data suggest that the diurnal variation in plasma 17-OH-CS levels and in the excretion of these compounds is a basic physiologic phenomenon. Since the change in urinary output follows that in plasma levels by about the same interval as found when cortisol is injected intravenously (4), it would appear that the cycle is primarily related to production of adrenal steroids rather than to alterations in their metabolism.

The plasma levels of free 17-OH-CS are a direct measure of the hormonal environment to which tissues are exposed at any given time. The rate of excretion of the conjugated compounds in the urine is only an indirect reflection of this environment, since it is delayed in time and involves a

TABLE 7. PLASMA IRON LEVELS (μG./100 ML.) IN BLIND SUBJECTS

Subjects	Time			
	8 A.M.	8 P.M.	12 M.	8 A.M.
26	130	130	139	132
27	48	48	71	46
28	97	46	116	91
29	105	96	84	107
30	53	44	41	61
31	46	74	87	46

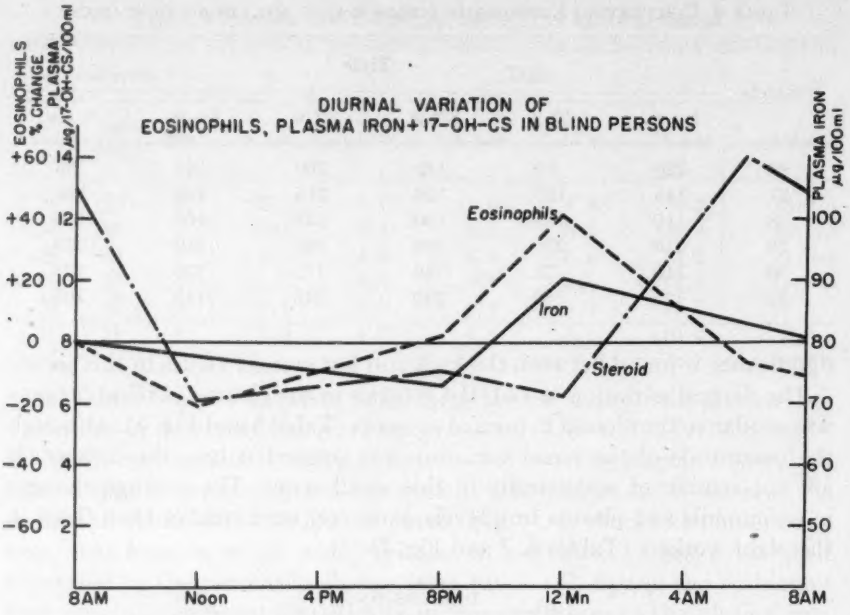

FIG. 7. Comparison of the mean variation of circulating eosinophils, plasma iron and plasma 17-OH-CS levels in the blind subjects.

series of intermediate reactions by which the hormone is converted into inactive compounds. The more rapid these reactions, the lower will be the plasma levels at any given rate of secretion from the adrenal. Although rate of metabolism must also be considered, therefore, in judging the activity of the gland, the actual hormonal environment in which the cells of the body function is represented best by the concentration of 17-OH-CS in the plasma.

That the relationships between urinary outputs of different individuals may actually be inverse to those between their plasma levels is suggested by the data on Subjects 16, 17, 18 and 19 (Figs. 3 and 4). In the first 2, the plasma concentrations were high and the output of conjugated products was low, whereas in Subjects 18 and 19 plasma levels were lower and the rates of excretion were high. Thus urinary values, although roughly reflecting the total adrenal secretion, are not a reliable measure of the hormonal environment, particularly with respect to time and in comparing individuals. A similar pattern has been observed in the plasma concentrations of conjugated dehydroepiandrosterone (12), in total 17-ketosteroid (17-KS) excretion (13, 14), and in the fraction of urinary 17-KS conjugated with glucuronic acid (4). The magnitudes of the differences in 17-KS are

somewhat smaller, however, than those of urinary 17-OH-CS. The failure of Pincus *et al.* (2) to find an exact proportionality between reducing lipids and 17-KS may well be related to differences in the rate of metabolism of the various compounds rather than to differences in secretion or in the hormonal environment.

A diurnal variation somewhat comparable to that in man has also been observed in the plasma 17-OH-CS levels of the rhesus monkey (15). On the other hand it is not so marked in the dog. Thus it seems to be related to the later stages of phylogenetic development.

Neither the mechanism nor the significance of the diurnal variation of steroid levels is understood. The present study demonstrates that muscular activity is not a direct factor. It must be pointed out, however, that the night workers studied here did not have an entirely regular schedule; they usually worked five consecutive nights a week, but were free on the remaining two nights. This must be considered in interpreting our findings. Our data also show that lack of ocular vision does not change the pattern of diurnal variation of plasma 17-OH-CS. The mechanism for this variation has not been determined.

Other constituents of plasma, such as iron (11) and circulating leukocytes, have been shown to vary diurnally. In the night workers these variations were, on the average, about the same in character and magnitude as has been observed in individuals during a more usual schedule of activity and sleep. The changes in the level of plasma iron are of special interest, because other workers (16, 17) have reported that changes in activity and sleep patterns result in a correlated shift in the diurnal variation of this factor. This would suggest that the regularity of reversed activity in our night workers was not sufficient to result in a shift in their diurnal patterns. Further study of this problem is in progress in our laboratory.

The relation of the variations in iron to those of steroid has been a subject of previous speculation (11). The lag in time (6 A.M. to 9 A.M.) between the peak values for plasma 17-OH-CS and plasma iron is about the same as the lag between the maximum of other metabolic effects after a peak in plasma steroids (18, 19). However, most processes such as infection or trauma, which are associated with adrenocortical stimulation, cause a fall rather than a rise in the level of plasma iron. In addition, the failure of exogenous corticotropin (19) or cortisone to affect plasma iron levels significantly, and the occurrence of a normal rhythm in this component in untreated Addison's disease (11), indicates the independence of these two diurnal changes. Our observation of negligible variation in the concentration of plasma iron in blind subjects, in whom the steroid changes were present, confirms this impression.

The variations in circulating eosinophils in the data presented are with-

out statistical significance. This reflects the very labile character of eosinophil changes and their sensitivity to non-adrenocortical influences. None the less, it seems probable that the usual daytime rise in eosinophils reflects, at least in part, the morning fall in 17-OH-CS levels.

SUMMARY

1. A diurnal rhythm in the levels of plasma 17-hydroxycorticosteroids of normal subjects has been demonstrated. A maximum value was regularly observed during the later hours of sleep (5 to 8 A.M.), usually near 6 A.M. From 8 A.M. to midnight the levels followed a downward trend, the decrease being more rapid in the first few hours after the peak. Between 2 A.M. and 6 A.M. the concentrations rose rapidly.

2. 17-Hydroxycorticosteroid excretion in urine followed the same general curve of variation as that of plasma, but was delayed about two hours.

3. Night workers and blind persons were found to have the same diurnal variation as the normal subjects.

4. In normal subjects and in the night workers plasma iron concentrations manifested a periodicity like that of the 17-hydroxycorticosteroids, but the cycle was two hours later. The cycle was not so definite in the blind individuals.

5. An inverse cycle seemed to exist in concentrations of eosinophils, but individual variation was so great that there is a considerable degree of statistical uncertainty as to the significance of these variations.

Acknowledgments

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CORTISONE THERAPY OF RHEUMATIC FEVER: RELATION OF CLINICAL RESPONSE TO LEVELS OF SERUM PROTEIN-BOUND IODINE, SERUM CORTICOIDS AND URINARY 17-KETOSTEROIDS*

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THE clinical and laboratory data on a group of 53 children ranging in age from 4 to 15 years and treated for initial attacks of rheumatic carditis with large amounts of cortisone have been presented previously (1, 2). The return of the abnormal cardiologic findings to normal in children with earlier unequivocal evidences of myocarditis seemed to depend upon the duration of illness prior to treatment; at present 12 of the 14 treated within two weeks of onset have normal hearts, and 16 of the 27 treated two to six weeks after onset have no cardiac abnormalities, with 3 others having only non-transmitted grade I systolic apical murmurs. This favorable outcome contrasts sharply with the finding that only 1 of the 14 treated after six weeks of illness now has a normal heart. The follow-up period extends from three to thirty-nine months.

In an effort to explain the differences in the post-therapy cardiologic findings in children with rheumatic carditis treated with large amounts of oral cortisone (1, 2), changes in the levels of serum protein-bound iodine, serum corticoids, blood eosinophils, and neutral urinary 17-ketosteroids were studied. Data on additional patients are included by virtue of treatment of recurrent attacks of rheumatic fever. In contrast to the oral regimen of cortisone employed in the original series (1, 2), 17 patients received intramuscular cortisone and 12 others were given adrenocorticotropic hormone intramuscularly.

METHODS

Samples of venous blood and 24-hour collections of urine were obtained for analysis prior to, during, and after treatment whenever possible. Urine was preserved either with

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glacial acetic acid or thymol. Serum was analyzed for protein-bound iodine by the method of Barker (3) as modified in this laboratory (4). Corticoids were measured by the method of Nelson and Samuels (5), using the Porter and Silber end-point (6). Direct total eosinophil counts in blood were made by the method of Randolph (7). The urinary neutral 17-ketosteroid excretion was determined by a method slightly modified from that of Drekter *et al.* (8). All analyses were made in duplicate.

RESULTS

I. *Serum protein-bound iodine levels prior to, during and following cortisone therapy*

The pretreatment concentrations of serum protein-bound iodine (PBI) were within normal limits (3.5–7.5 gamma per 100 cc.) in all but a few of this group of children with acute rheumatic myocarditis. These levels decreased in the course of cortisone therapy during all but the two weeks with the smallest numbers of observations—the fifth and seventh (Fig. 1). In 9 patients levels between 3.4 and 1.5 gamma per 100 cc. were recorded during the treatment period. Only 1 patient showed a rise during this interval. The decrease in the mean values of serum PBI noted during

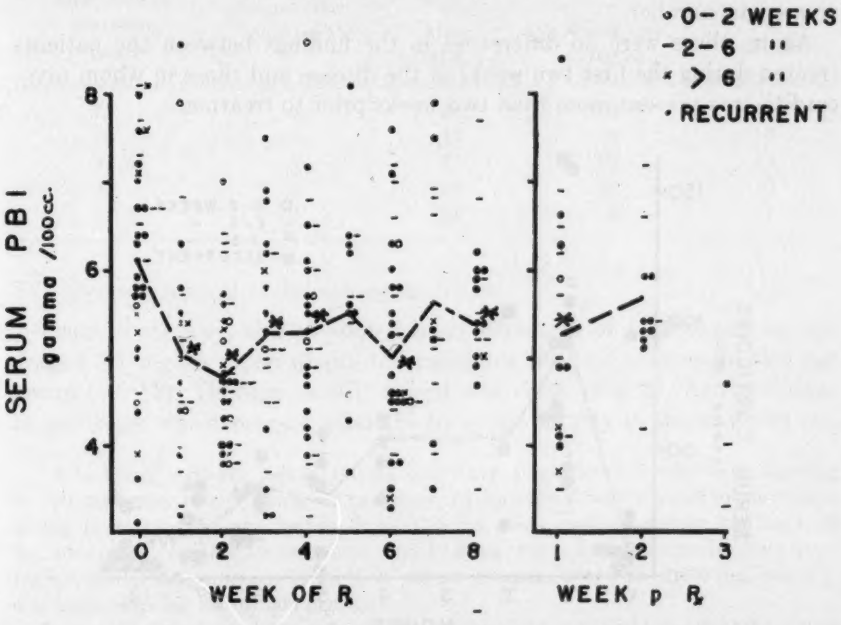

FIG. 1. Serum protein-bound iodine concentrations in children with rheumatic fever prior to, during and following cortisone therapy. Duration of illness at onset of treatment is indicated by variation in symbol. The dotted line denotes mean values. Asterisks indicate significant deviation from pretreatment values.

therapy persisted for approximately one week following withdrawal of the drug.

Separation of the patients into groups on the basis of duration of illness prior to therapy—less than two weeks, from two to six weeks, and more than six weeks (see symbols in Figure 1)—or on the basis of outcome—complete resolution or presence of residual myocardial alterations—showed no correlation with the changes in serum protein-bound iodine concentration. None of the patients showed any clinical signs of hypothyroidism.

II. *Blood eosinophil counts*

The blood eosinophil cell counts were equally decreased, irrespective of the duration of the illness prior to therapy. Institution of the regimen which included cortisone had practically no effect upon the eosinophil counts; therefore these results are not shown. Statistically significant differences in the decreases were noted only during three of the twenty-eight weeks of observation.

In the first week of the post-therapy period the blood eosinophils increased in number.

Again, there were no differences in the findings between the patients treated during the first two weeks of the disease and those in whom myocarditis was present more than two weeks prior to treatment.

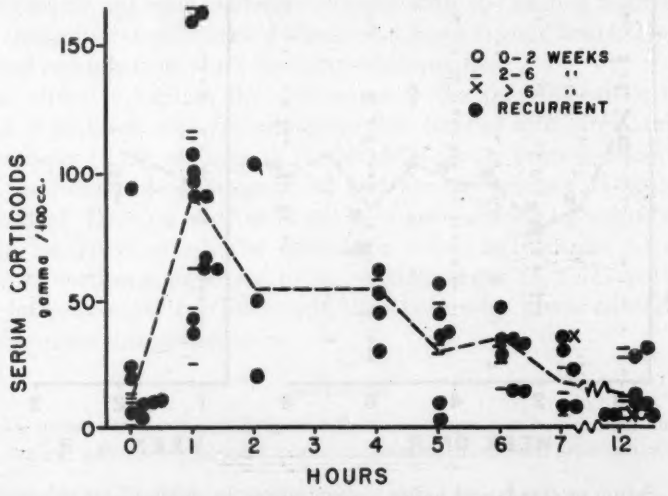

FIG. 2. Serum corticoid values after ingestion of 100 mg. of cortisone. Similar concentrations are noted, regardless of duration of illness before treatment.

III. *Serum corticoid levels*

With 1 exception, the pretreatment concentrations of serum corticoids were within normal limits as defined in this and other laboratories (9–11), ranging from 0 to 23 gamma per 100 cc. Patient M.H., who had severe joint pains and high fever in addition to myocarditis, had an initial level of 95 gamma per 100 cc. Following one week of bed rest and salicylate therapy without cortisone the value declined to 25 gamma per 100 cc.

Serum corticoid levels at intervals following ingestion of 100 mg. of cortisone are also shown in Figure 2. One hour after receiving 100 mg. of cortisone the serum corticoid levels increased to an average value of 90 gamma per 100 cc. and gradually returned to the pretreatment range within seven hours. This response was the same, irrespective of the duration of the illness prior to therapy. In doses of less than 100 mg. the oral cortisone usually had less effect on the serum corticoid levels (Table 1).

TABLE 1. SERUM CORTICOID LEVELS FOLLOWING LESS THAN 100 MG. OF ORAL CORTISONE

Oral dose of cortisone (mg.)	Hours after cortisone	Serum corticoid concentration		
		Mean (γ/100 cc.)	±Stand. dev. (γ/100 cc.)	No.
75	6	22	22	14
50	6	25	28	16
37.5	5	27	19	15
25	4	34	38	16
12.5	2	23	14	17

IV. *Urinary neutral 17-ketosteroid excretion*

Prior to therapy, the 24-hour urinary excretion of 17-ketosteroids averaged 3.1 mg., a value within the range for healthy controls in this age group (12, 13). Therapy usually raised this value (Fig. 3). As the dosage of cortisone[1] was decreased after the forty-second day of therapy, the uri-

[1] Schedule of cortisone dosage: 100 mg. orally every eight hours for six weeks, tapering to 100 mg. every twelve hours for two days, 75 mg. every twelve hours for two days, 50 mg. every twelve hours for two days, 37.5 mg. every twelve hours for two days, 25 mg. every twelve hours for two days, and 12.5 mg. every twelve hours for four days. Intramuscular cortisone was given to 17 others in amounts of 100 to 500 mg. per day; 4 of them received 300 to 500 mg. daily.

Intramuscular adrenocorticotropic hormone was given to 12 children; 10 of them were given 25 units every six hours; 1 received 10 units every six hours; and 1 was given 40 units once a day.

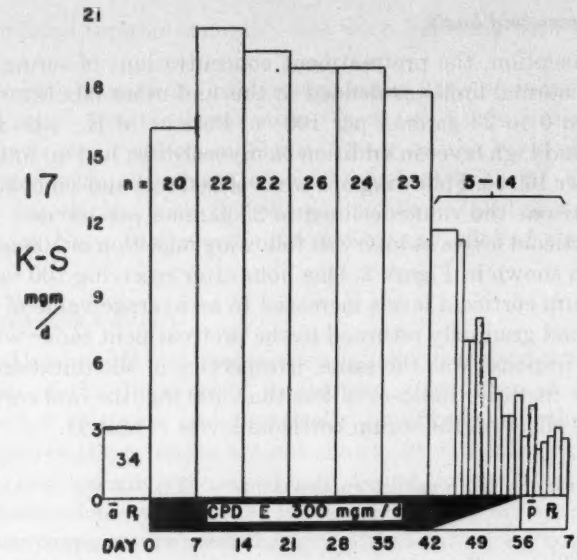

FIG. 3. Urinary 24-hour excretion of 17-ketosteroids before, during and after cortisone therapy.

nary excretion of 17-ketosteroids decreased progressively and reached pretreatment levels by the fifty-fourth day. At this time the patients were receiving approximately 25 mg. of the steroid each day. There was no further drop in the post-therapy period.

TABLE 2. COMPARISON OF 24-HOUR URINARY 17-KETOSTEROID EXCRETION FOLLOWING ORAL AND INTRAMUSCULAR CORTISONE IN CHILDREN WITH RHEUMATIC CARDITIS

	Oral cortisone (300 mg./d)				I.M. cortisone (300 mg./d)		
	Day	Mean (mg./d)	±Stand. dev.	No.	Mean (mg./d)	±Stand. dev. (mg./d)	No.
Pre R_X		3.1	1.8	34			
R_X	7	16.1*	12.7	20	5.7†	4.6	17
	14	20.4*	19.7	22	6.3†	3.9	14
	21	19.4†	21.3	22	6.6†	2.6	6

* Deviation from value following oral cortisone is statistically greater than the one after intramuscular cortisone.

† Deviation from pretreatment mean is statistically significant.

The increases following oral cortisone were several times greater than those following intramuscular cortisone (Table 2).

Inspection of the serum corticoid and urinary 17-ketosteroid data, arranged by groups in accordance with the duration of the disease prior to therapy (less than two weeks, from two to six weeks and more than six weeks), again revealed no difference between those who responded with resolution of the carditis and those who were left with residual cardiac damage.

DISCUSSION

Therapy of rheumatic carditis with cortisone or corticotropin resulted in the drop in serum PBI level, the rise in serum corticoid level, the increase in urinary output of 17-ketosteroids, and the decrease in blood eosinophils described by others (9–14, 18–36). Our studies indicate that neither the presence of these changes nor their intensity has any relationship to the clinical outcome, *i.e.*, there is no correlation of these factors with ultimate resolution or non-resolution of the carditis.

The greater rise in urinary 17-ketosteroid excretion after oral than intramuscular cortisone is in keeping with the report of Fajans *et al.* (37). This difference occurred despite similar dosage schedules. There are several possible explanations. One is that cortisone is converted into neutral 17-ketosteroids in the liver, where the administered steroid is found in large concentrations immediately after absorption through the gastro-intestinal tract. Another reason would be destruction or inactivation of cortisone at the intramuscular site. In addition, greater urinary excretion of cortisone administered intramuscularly could account for a large loss of the steroid. Hellman *et al.* have shown that 70–80 per cent of intravenously administered hydrocortisone-4-C^{14} is eliminated in the urine in twenty-four hours and slightly more than 90 per cent in the urine and feces in seventy-two hours (38).

The slight but significant decline in serum levels of protein-bound iodine is presumably related in part to decreased synthesis of hormone as a consequence of an intrathyroidal block (39). It is also recognized that cortisone increases the urinary output of iodide and thereby diminishes uptake. Likewise, the changes in protein concentration which occur during cortisone therapy of rheumatic fever (2) may affect the binding of thyroxine to alpha globulin (40). It cannot be attributed to a decreased output of pituitary thyrotropic hormone (21) nor to an increased peripheral destruction of the circulating protein-bound iodine (41).

SUMMARY

1. Children with initial or recurrent attacks of rheumatic carditis showed, on the average, normal levels of urinary neutral 17-ketosteroids,

serum corticoids and serum protein-bound iodine prior to therapy with cortisone or corticotropin. The blood eosinophil counts were low.

2. The serum protein-bound iodine level decreased slightly during cortisone or corticotropin therapy, but no patient showed any clinical evidence of thyroid hypofunction.

3. Cortisone therapy, orally or intramuscularly, resulted in an increase in urinary neutral 17-ketosteroids. The rise was greater following oral administration. Corticotropin in large dosage for three to six days at the end of cortisone treatment did not maintain this rise.

4. The serum corticoid level was normal prior to cortisone therapy and increased during treatment. Peak values were recorded at one hour; high values, though at a decreased level, were still present six hours following oral administration of the steroid.

5. The eosinophil count, already low prior to therapy, rarely decreased further.

6. The changes in serum protein-bound iodine and corticoid levels, urinary 17-ketosteroids, and blood eosinophils during therapy were of the same orders of magnitude, irrespective of the duration of the illness or the degree of resolution of the carditis.

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EFFECT OF PREDNISONE AND PREDNISOLONE ON THYROID FUNCTION, WITH SPECIAL REFERENCE TO THYROXINE-BINDING PROTEIN IN NEPHROSIS

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INTRODUCTION

ACTIVATION of the adrenal cortex by corticotropin and the administration of adrenal steroid hormones profoundly influences thyroid activity (1–4), the level of circulating thyroid hormone (5–7), and the renal clearance of iodine by the kidney (8–10). This paper is concerned with the results of experiments designed to demonstrate and elucidate the mechanisms whereby prednisone and prednisolone influence thyroid activity in man.

METHODS AND MATERIAL

Subjects

The present studies were carried out on 14 patients. They were clinically euthyroid although 1 of them had a highly active remnant of thyroid gland following thyroidectomy performed for thyrotoxicosis ten years previously. The patients had been admitted to the Clinical Center of the National Institutes of Health for study of diverse disease states such as rheumatoid arthritis, carcinoma of the breast, scleroderma, cirrhosis, lupus erythematosus disseminatus, nephrosis and dermatomyositis.

Methods

Thyroidal radioactive iodine (I^{131}) uptake was determined by direct counting over the gland, using a scintillation counter. The determination consisted of ascertaining the fraction of a 10-microcurie dose, orally administered in water, which had been picked up by the thyroid at the end of twenty-four hours.

Serum was analyzed for protein-bound iodine (PBI) concentration by the method of Zak *et al.* (11) as modified by Leffler (12). Normal values range from 4 to 8 gamma per 100 ml. Serial determinations of serum PBI concentration, by this method, are shown to be constant in euthyroid subjects; the maximal variation over a period of months usually does not exceed 1.0 gamma per 100 ml.

Thyroidal secretion rates were determined as described by Goldsmith *et al.* (13), except that larger doses of Tapazole® were employed to assure complete block of hormone formation from recirculated I^{131} resulting from the peripheral breakdown of I^{131}-labelled

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hormone released from the gland. A 200-microcurie dose of I^{131} was administered orally in water. Twenty-four hours later Tapazole® was started in a dosage of 30 mg. every eight hours by mouth; thereafter, a daily count was made over the neck. With correction for the physical decay of I^{131}, the counts per minute over the gland were plotted semilogarithmically versus the time in days. A straight-line function ensued which graphically demonstrated the rate of release of hormonally bound I^{131} from the thyroid gland. Control and experimental periods could thus be incorporated into a single study on the same patient over a period of two to three weeks.

The basal metabolic rates were determined by the Benedict-Roth technique. The methods for serum cholesterol and total protein concentration were those employed by the Clinical Center routine chemistry laboratory.

Materials

Prednisone (Meticorten®) and prednisolone (Meticortelone®) were administered orally in divided dosages of from 20 to 80 mg. per day.

Cortisone acetate was administered orally in a divided dosage of 250 mg. per day.

Hydrocortisone was administered orally in a divided dosage of 160 mg. per day.

A 10-unit (25 mg.) dose of thyrotropin (TSH) was administered intramuscularly in saline solution to 1 patient.

Tapazole® was administered orally in a dosage of 30 mg. every eight hours.

Radioactive iodine (I^{131}) was obtained from the Oak Ridge National Laboratories, Oak Ridge, Tennessee and was administered orally in doses of 10 to 200 microcuries, carrier-free, and dissolved in water.

RESULTS

a. *Prednisone-treated patients, excluding patients with nephrosis (Table 1)*

Serum PBI levels, determined in 8 patients, were found to be decreased in 6 and unchanged in 2. In 4 patients, following the maximal drop in the serum PBI level there was a gradual rise which, however, did not reach the control values while therapy was continued. The maximal fall in serum PBI concentration was usually reached in three to four weeks.

Thyroidal 24-hour uptakes of I^{131}, determined in 8 patients, were found to be decreased in 6 and essentially unchanged in 2. In 1 patient there was a rebound to an uptake higher than that of the control value, when the 24-hour I^{131} uptake was measured five days after therapy was stopped.

The thyroidal secretion rate (Fig. 1), determined in 1 patient, was depressed from a control half-life of 5 days to a half-life of 8.5 days. This returned to the control rate of secretion within twelve hours after therapy was stopped.

Basal metabolic rates, measured in 4 patients, were unchanged in 3 and depressed in 1.

Serum total protein concentrations, measured in 6 patients, were unchanged in 4, and decreased slightly in 2.

Serum total cholesterol concentrations, measured in 5 patients, were increased in 3, slightly increased in 1, and unchanged in 1.

TABLE 1. THE EFFECT OF PREDNISONE (METICORTEN) ON THYROID FUNCTION

Patient	Day	Serum PBI (μg./100 ml.)	Thyroidal I^{131} uptake (%)	BMR (%)	Serum cholesterol (mg./100 ml.)	Serum tot. protein (Gm.%)	Medication*
T.T., 42WM	1	5.3	30.7	—	—	6.0	None
Cirrhosis	10	4.1	21.4	—	—	—	9d., 50 mg./d.po
	16	3.75	16.0	—	—	6.1	15d., 50 mg./d.po
	21	3.6	60.7	—	—	—	2d., no medication
P.M., 19 WM	1	6.3	50.0	—	—	6.2	None
Touraine-Solente-Jole syndrome	7	5.1	22.0	—	—	7.2	8d., 60 mg./d.po
	20	3.7	17.7	—	—	6.0	4d., hydrocortisone, 160 mg./d.po
	29	4.0	30.0	—	—	6.1	2d., no hydrocortisone
C.B.C., 47WM	1	5.5	45.5	—	111	7.0	None
Rheumatoid arthritis	11	5.0	—	—	—	—	10d., 40 mg./d.po
	17	4.9	37.0	—	125	7.1	17d., 40 mg./d.po
	25	5.5	39.0	—	122	6.9	2d., 20 mg./d.po
	31	5.45	—	—	128	7.1	2d., no medication
E.B., 53 WF	1	5.6	16.0	+ 4	137	7.9	None
Carcinoma of breast	14	4.25	—	—	200	7.8	5d., 50 mg./d.po
	25	3.85	15.0	− 2	188	7.0	16d., 50 mg./d.po
	35	4.6	—	—	216	6.9	26d., 50 mg/.d.po
	46	3.85	24.0	+ 2	236	6.3	37d., 50 mg./d.po
	53	3.0	—	—	204	6.6	44d., 50 mg./d.po
	57	3.8	18.0	+ 3	192	6.3	48d., 50 mg./d.po
C.D., 45 WM	1	5.6	69.0	—	230	—	None
Post-thyroidectomy (10 yrs.)	7	3.0	40.0	—	—	—	6d., 60 mg./d.po
	14	3.7	68.0	—	220	—	6d., no medication
	28	3.75	69.6	—	237	—	19d., no medication
	32	4.4	65.4	—	221	—	23d., no medication
M.J., 33 NF	1	4.65	32.0	−12	99	8.6	None
Lupus erythematosus	14	3.35	19.4	—	96	—	5d., 30 mg./d.po
	22	3.5	17.9	− 6	129	7.6	12d., 30 mg./d.po
	29	5.0	14.5	−15	144	7.5	6d., 20 mg./d.po
	37	5.1	20.3	—	151	7.1	14d., 20 mg./d.po
A.H., 60 WM	1	4.2	36.0	−19	225	6.7	None
Scleroderma	5	4.7	—	− 9	234	—	4d., 30 mg./d.po
	16	4.3	20.0	−11	212	—	13d., 30 mg./d.po
	20	4.5	22.0	−14	216	—	17d., 30 mg./d.po
	30	5.25	15.7	−14	215	6.1	6d., 40 mg./d.po
	35	4.2	17.0	−18	202	5.9	11d., 40 mg./d.po
	42	3.05	22.1	−15	218	—	6d., 30 mg./d.po
	49	3.7	—	−15	248	6.0	13d., 30 mg./d.po
R.B., 50 WF	1	6.85	47.0	+ 3	252	7.0	None
Rheumatoid arthritis	7	6.6	33.0	− 4	284	—	6d., 30 mg./d.po
	15	5.25	32.0	−13	239	6.6	14d., 30 mg./d.po
	22	4.25	31.0	−13	278	6.2	21d., 30 mg./d.po
	29	2.75	—	−13	291	—	28d., 30 mg./d.po
	36	5.25	27.4	−13	298	—	35d., 30 mg./d.po
	44	6.0	—	−19	300	6.6	43d., 30 mg./d.po

* Meticorten, unless otherwise noted.

b. *Prednisolone-treated patients* (*Table 2*). Serum PBI levels were determined in 2 patients and found to be decreased in both. In 1 patient, after cessation of therapy, there was a rise in serum PBI concentration to a level above the control value.

Thyroidal 24-hour I^{131} uptakes, determined in 2 patients, were decreased

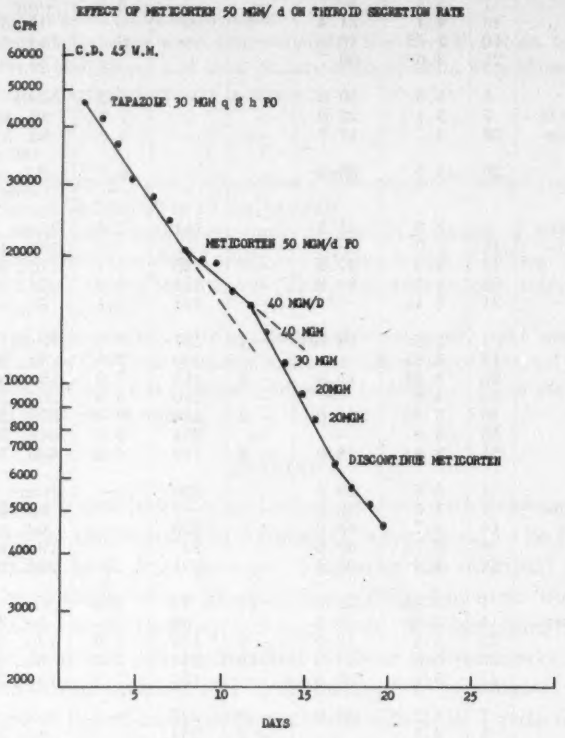

FIGURE 1

in both. One patient showed a rise in 24-hour uptake above the control value after therapy was stopped.

The thyroidal secretion rate (Fig. 2), determined in 1 patient, was depressed from a control half-life of 4 days to a half-life of 7.5 days. After a repeat control period following cessation of prednisolone therapy, cortisone acetate was administered and the half-life was depressed to eleven days. During the cortisone treatment period, a dose of 25 mg. of thyrotropin was administered intramuscularly and a prompt marked discharge of hormonally bound I^{131} from the gland was demonstrated.

Serum total protein concentrations, measured in 2 patients, were unchanged in both.

Serum total cholesterol concentration was increased in the 1 patient in whom it was measured.

Summary. It would appear that prednisone and prednisolone, administered to subjects with normal thyroidal and renal functions, consistently induce a fall in the serum PBI level, a depression of the thyroidal 24-hour

TABLE 2. THE EFFECT OF PREDNISOLONE (METICORTELONE) ON THYROID FUNCTION

Patient	Day	Serum PBI (mg./100 ml.)	Thyroidal I^{131} uptake (%)	Serum cholesterol (μg./100 ml.)	Serum tot. protein (Gm. %)	Medication
A.H., 62 WF Carcinoma of breast	1	4.8	32.0	186	4.1	None
	10	4.8	17.0	—	5.4	9d., 50 mg./d.po.
	18	4.4	—	252	5.4	17d., 50 mg./d.po
	24	3.95	15.2	—	5.5	23d., 50 mg./d.po
	30	3.6	—	223	5.3	29d., 50 mg./d.po
	39	3.25	14.0	244	5.1	38d., 50 mg./d.po
B.M., 18 WM Myelofibrosis	1	5.0	22.0	—	8.1	None
	7	3.7	10.0	—	—	6d., 60 mg./d.po
	13	4.6	16.6	—	8.0	2d., 40 mg./d.po
	17	5.4	21.0	—	—	3d., 30 mg./d.po
	28	6.5	32.8	—	7.9	10 d., no medication

I^{131} uptake, and a slowing of thyroidal hormonally bound I^{131} secretion. No consistent change in the serum total protein level or in the basal metabolic rate was demonstrated and the serum total cholesterol concentration tended to increase with therapy. In 1 patient, cortisone was also effective in depressing the thyroidal secretion rate. Moreover, it was demonstrated that the ability of the thyroid gland to respond to exogenous thyrotropin by increasing the release of hormonal I^{131}, was unimpaired while the secretion rate was depressed by cortisone.

c. *Prednisone-treated patients with nephrosis* (*Table 3*). Three patients with nephrosis were treated with prednisone in a dosage of 20–80 mg. per day orally, for about sixty-five days.

Serum PBI concentration in all 3 patients was low-normal before therapy was started. In 2 patients an initial depression in the serum PBI level occurred two to three weeks after therapy was started; in 1 there was no decrease. Following the initial decrease in the concentration of serum PBI, and paralleling a fall in the serum total cholesterol level and a rise in the

total protein level concurrent with clinical improvement, there was a rise in serum PBI concentration far above the initial control value in all 3 patients. In 2 of them, after the nephrosis was markedly improved, continued administration of prednisone resulted in a fall in the serum PBI level toward the initial low control value.

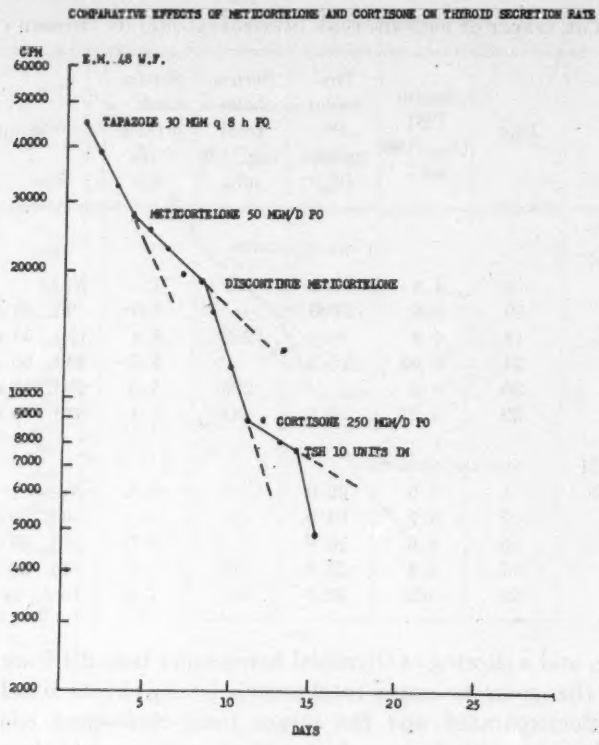

FIGURE 2

Thyroidal 24-hour I^{131} uptakes, determined in 3 patients, were markedly depressed in 2 and slightly depressed in 1.

Serum total protein concentrations, measured in 3 patients, were elevated toward normal in all the patients during the course of therapy.

Serum total cholesterol concentrations, measured in 3 patients, gradually fell to normal from control high values.

Summary. It would appear that prednisone, administered to nephrotic patients, does not induce the depression of serum PBI concentration noted in normal patients. Concurrent with regression of the nephrotic state the fall in serum cholesterol level, rise in serum total protein level, and loss of

TABLE 3. THE EFFECT OF PREDNISONE (METICORTEN) ON THYROID FUNCTION IN NEPHROTIC PATIENTS

Patient	Day	Serum PBI (μg./100 ml.)	Thyroidal I^{131} uptake (%)	Serum cholesterol (μg./100 ml.)	Serum tot. protein (Gm. %)	Medication*
E.C.W., 50CM	1	3.0	51.0	976	3.8	None
	9	2.45	34.4	1274	3.9	7d., 40 mg./d.po
	20	1.35	34.3	965	4.1	18d., 40 mg./d.po
	26	2.6	41.0	835	5.0	24d., 40 mg./d.po
	33	6.25	40.1	570	5.2	31d., 40 mg./d.po
	42	6.75	45.0	413	5.4	40d., 40 mg./d.po
	47	6.9	46.5	307	6.0	45d., 40 mg./d.po
	56	7.75	44.2	304	6.3	54d., 40 mg./d.po
	64	7.2	—	256	5.8	62d., 40 mg./d.po
	70	6.35	43.3	—	6.0	68d., 40 mg./d.po
S.K.S., 29CM	1	3.6	45.0	929	3.4	None
	7	2.0	—	—	3.6	None
	14	2.1	—	888	3.7	None
	19	3.0	20.0	915	4.1	5d., 40 mg./d.po
	26	3.5	14.7	343	5.3	12d., 40 mg./d.po
	42	6.5	12.9	416	5.4	28d., 40 mg./d.po
	50	7.7	22.9	356	6.1	35d., 40 mg./d.po
	54	7.4	17.3	384	5.8	40d., 40 mg./d.po
	64	4.95	—	375	5.8	8d., 80 mg./d.po
	70	5.85	14.5	423	6.1	14d., 80 mg./d.po
O.S.H., 39WM	1	4.0	52.0	519	4.6	None
	12	5.7	—	485	—	1d., 20 mg./d.po
	19	4.2	33.0	465	4.3	7d., 20 mg./d.po
	21	4.1	23.0	439	—	2d., 40 mg./d.po
	33	5.1	30.2	474	4.5	14d., 40 mg./d.po
	41	6.6	27.0	484	—	22d., 40 mg./d.po
	49	4.5	26.8	389	4.0	6d., hydrocortisone, 160 mg./d.po
	54	4.7	27.1	330	5.2	11d., hydrocortisone, 160 mg./d.po
	64	4.25	—	463	4.9	21d., hydrocortisone, 160 mg./d.po
	70	3.75	28.7	503	5.3	5d., 40 mg./d.po

* Meticorten, unless otherwise noted.

edema, there is a marked rise in the concentration of serum PBI. This occurs despite continued prednisone administration which, in patients without nephrosis, induces a progressive decrease in the level of serum PBI.

DISCUSSION

Corticotropin, cortisone (1–7) and, in the present study, prednisone and prednisolone have been demonstrated to cause a decrease in serum PBI concentration, thyroidal 24-hour I^{131} uptake, and thyroidal secretion rate (2). The exact mechanisms whereby these steroids cause these changes are subject to much controversy (1–6, 8, 9).

Three possible mechanisms have been suggested (2): a) increased renal clearance of I^{131} (8) as the cause of the decreased 24-hour I^{131} uptake; b) decreased sensitivity of the thyroid gland to endogenous thyrotropin when under the influence of adrenocortical steroids (14, 15); or c) decreased rate of secretion of thyrotropin by the pituitary gland when under the influence of adrenocortical steroids (2, 5).

The 24-hour radioiodine uptake as a measure of thyroid function is subject to many extra-thyroidal influences (1, 7). In animals and humans, corticotropin and adrenal steroids are known to promote renal excretion of iodide (8–10); therefore, tests based solely on the thyroidal uptake of I^{131} or the urinary excretion of I^{131} are unreliable indices of thyroid function during the administration of corticotropin or adrenocortical steroid (2).

Cortisone and corticotropin are reported to reduce the increase in thyroidal I^{131} uptake induced by injection of thyrotropin in the hypophysectomized rat (15–17). These results are open to criticism, since changes in renal excretion may have influenced iodine uptake by the thyroid. Ingbar (8) has shown that cortisone induces increased renal I^{131} excretion in hypophysectomized rats and that this is sufficient to account for the apparent reduction in I^{131} uptake in rats treated with thyrotropin plus cortisone as compared to thyrotropin alone. Thus, an alteration in the ability of the thyroid to respond to thyrotropin does not provide an adequate explanation for the effects of corticotropin, cortisone, prednisone and prednisolone on thyroid function.

The measurement of the release of I^{131} from the thyroid gland affords a better index of thyroid activity than does measurement of I^{131} uptake. The release of I^{131} from the gland is directly related to the hormonal secretion, in that loss of I^{131} from the gland is due to the loss of I^{131}-labelled thyroid hormone (2, 12). The factors of renal loss of I^{131} (7, 8), and changes in iodide space (1) and blood iodide content (1, 2) do not influence the rate of release of I^{131} to any appreciable extent. Measurement of the rate of release of I^{131} provides a continuous measure of thyroid activity, allowing control and experimental periods to be incorporated in a single study (2, 12). From the studies of the secretion rate in man reported in this paper and from similar

studies carried out in rabbits (2, 18), it seems probable that the adrenocortical steroids suppress thyroid function by suppressing the secretion of thyrotropin. In man and rabbits thyrotropin has been shown to be effective in increasing the thyroidal discharge of hormonally bound I^{131} in spite of the concurrent depression of the thyroidal secretion rate by cortisone.

The rise in serum PBI concentration observed in nephrotic patients during administration of adrenocortical steroids raises further questions as to the mechanism of the changes in serum PBI under the influence of various steroid hormones. Both in this study and that of Hill *et al.* (5), nephrotic patients demonstrated a markedly different pattern of change in the serum PBI content during steroid hormone therapy. Concurrent with a return of the serum total protein concentration to a normal level, there was a rise in serum PBI concentration to a value far above the control level. Recant and Riggs (19) found that the loss of iodine with protein in the urine did not adequately explain the low levels of serum PBI observed in nephrotic patients. They felt that the low protein level in nephrotics furnished insufficient transport protein for the thyroid hormone. The rise in serum PBI concentration, paralleling the rise in serum total protein concentration in nephrotic patients during adrenocortical steroid therapy, strongly suggests that alterations in the concentration of specific thyroxine-binding proteins (alpha-2 globulins) may be induced by the steroid hormone medication which increases the transport ability of the serum for thyroxine (20).

In pregnancy and in patients receiving estrogen therapy, the rise in serum PBI concentration may be due to an increased thyroxine-binding capacity of the blood proteins (21).

SUMMARY

1. The effects of prednisone (Meticorten) and of prednisolone (Meticortelone) on thyroid function have been studied in 14 patients with diverse diseases.

2. Prednisone and prednisolone administered to subjects with normal thyroid and kidney function consistently induce a depression in serum PBI level, thyroidal 24-hour I^{131} uptake, and rate of secretion of hormonally bound I^{131}.

3. The ability of the thyroid gland to respond to administered thyrotropin is unimpaired when the thyroidal secretion rate is depressed by cortisone.

4. In nephrotic patients there is a rise in the level of serum PBI, paralleling that of the serum total protein. This is in contradistinction to the usual fall in serum PBI concentration observed in normal subjects during adrenocortical steroid hormone therapy. Such a seemingly paradoxic response can be explained by a rise in serum thyroxine-binding protein that occurs in nephrotic patients as their disease regresses.

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STUDIES ON OBESITY. III. EFFECT OF ASCORBIC ACID ON THE INSULIN-GLUCOSE TOLERANCE CURVE

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THE role of ascorbic acid during stress has been emphasized by several investigators (1). Stress not only depletes the body of ascorbic acid but augments the requirements (1, 2). Dugal *et al.* (3, 4) have shown that pretreatment with ascorbic acid will prevent the typical enlargement of the adrenals in the guinea pig when under the influence of stress. These and other experiments (5, 6) indicate that ascorbic acid plays a compensatory role somewhat similar to the one of the adrenocortical hormones, and perhaps prevents release of adrenocorticotropin by the anterior pituitary.

Menten, Sigal and King (7, 8) have shown that in the scorbutic guinea pig (which cannot synthesize vitamin C) there is reduced carbohydrate tolerance. This was evident even before external signs of scurvy were present and was enhanced by the addition of another stressor, namely, the injection of diphtheria toxin. The peak of the glucose tolerance curve moved up and to the right in successive stages of vitamin depletion and was reversible to a certain extent, with only slight change in the fasting blood sugar levels. Animals sacrificed in the prescorbutic stage showed hydropic degeneration of the beta cells of the islets of Langerhans; at a later stage arteriosclerotic changes were evident. Stewart *et al.* (9) investigated the effect of insulin in scorbutic and prescorbutic monkeys and found them relatively insensitive. They interpreted their data as indicating that adrenocortical function is increased rather than decreased in scurvy. Besides this direct effect on the adrenal cortex and the insulin-elaborating mechanism, there is evidence that ascorbic acid is important in the metabolism of fatty acids, and that its absence increases the synthesis of cholesterol (10–13).

Since obese patients are under continuous daily mechanical stress, of greater or lesser degree, it was felt that the body might be partly depleted of ascorbic acid despite an average vitamin intake; the depletion would be enhanced by the fact that the vitamin requirements of the obese must be greater than average, in view of a larger body mass. Analyses of the diets

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of our obese patients showed that, though the majority consumed food high in carbohydrate content, it was deficient in fruits and vegetables. There is wide disagreement as to the existence and manifestation of hypovitaminosis C, but it has been shown recently that blood levels consistent with such a state, or even frank scurvy, are not rare in the North American continent (14). The present study was undertaken to ascertain whether or not ascorbic acid would have any effect on restoring to normal the impaired carbohydrate metabolism of the obese. It was decided to study changes, if any, in the insulin-glucose tolerance curve rather than in the oral glucose tolerance curve, since it was felt that the former test would reflect earlier changes in carbohydrate tolerance. Previous studies on 32 obese patients carried out in our Research Unit showed that all but 1 had an abnormal insulin-glucose tolerance curve (15).

PROCEDURE

Insulin-glucose tolerance curves were obtained according to the procedure described by Engel and Scott (16). The subjects were fasted overnight and kept at rest the following morning. Venous blood was withdrawn and 0.1 unit of regular insulin per kilogram of body weight was injected intravenously. At 30 minutes , or when the first significant symptoms of hypoglycemia appeared (depending on which occurred first), a sample of blood was withdrawn and 0.8 Gm. of glucose per kilogram of body weight was given by mouth. Additional blood samples were taken at 60, 90, 120 and 180 minutes from the time of the initial sample. For comparative purposes, the blood sugar levels are reported as a percentage of the initial blood sugar level, which is taken as 100 per cent.

The patients were then given 500 mg. of ascorbic acid[1] orally every six hours for a minimum period of three weeks, but usually for an average of four weeks. The insulin-glucose tolerance test was then repeated, using the same amounts of insulin and glucose administered during the first test. In 6 patients the test was repeated after a similar period without ascorbic acid; they had been given a reducing diet to see whether a loss of 5 to 15 pounds would affect the tolerance curve.

Blood sugar levels were determined according to the Folin-Wu method adapted to the spectrophotometer after precipitating the proteins with 10 per cent sodium tungstate (17). Blood ascorbic-acid levels were determined according to the method of Heinemann and Hald (18).

RESULTS

The results of the insulin-glucose tolerance tests in 21 obese subjects before and after the oral administration of ascorbic acid are shown in Table 1 and in Figure 1. The degree of overweight varied from 30 to 130 per cent according to the ideal weights listed in the Davenport charts, using 35 years as the optimum age. The average degree overweight was 74.4 per cent and the average duration of the obese state, 14.5 years. All the patients in this study had an abnormal insulin-glucose tolerance curve, *i.e.*,

[1] Ascorbic acid was supplied through the courtesy of Ayerst, McKenna and Harrison, Limited.

TABLE 1. THE EFFECT OF ORAL ASCORBIC ACID ON THE INSULIN-GLUCOSE TOLERANCE OF 21 OBESE PATIENTS

Patient No.	Age and Sex	Weight change	% Initial blood sugar level					Insulin reaction
			30 min.	60 min.	90 min.	120 min.	180 min.	
Group I: Obese patients with abnormal oral glucose tolerance curve								
1	32F*	− 9	46	101	170	203	165	+
			51	123	169	183	134	+
2	32M	−14	72	161	197	169	143	−
			37	107	155	174	87	?
			48†	114	128	88	88	
3	40F*	0	52	98	166	147	141	−
			39	108	164	164	150	−
4	43M	−16	62	133	184	152	112	−
			53	132	183	168	109	−
5	44M	−12	50	123	202	225	98	−
			43	115	182	189	140	−
6	47M	?	74	114	178	187	135	−
			70	106	151	166	136	−
			73†	117	166	175	151	−
7	53M	− 6	47	113	185	211	124	+
			52	100	153	198	191	+
8	53M	0	37	112	229	216	137	−
			50	100	169	150	87	−
Avge.	43	− 8	Before 55	120	189	189	132	
			After 49	111	166	174	129	
			S.D. of differences 10.2	16.4	17.4	19.0	25.5	
			S.E. 3.59	5.8	6.16	6.7	9.0	
			Value of P <.02	<.05	<.02	<.01	<.01	
Group II: Obese patients with normal oral glucose tolerance curve								
9	15M*	0	64	163	163	152	113	−
			40	128	122	118	107	−
10	19F*	+ 1	32	139	150	127	116	−
			46	105	123	128	116	−
11	21F*	− 3	58	84	101	130	138	−
			67	122	153	153	129	−
12	32F*	− 6	47	136	167	163	110	++
			49	99	139	139	112	+
13	32F*	− 5	53	142	217	182	124	−
			28	113	141	147	97	−
14	33M	−10	44	175	219	201	91	−
			42	112	152	117	75	−
15	36M	− 5	52	132	193	198	127	−
			59	103	89	88	70	−
16	36F*	− 1	41	93	140	166	166	−
			34	103	135	152	152	−
17	37F*	?	42	79	119	144	123	−
			32	75	112	145	121	−
18	38F*	0	58	115	157	151	125	−
			54	86	146	139	125	−
19	44M	− 4	30	79	157	171	142	−
			44	80	90	131	112	+
20	45F*	− 8	40	88	161	167	153	++
			33	96	119	127	129	+
21	49M	− 1	53	100	137	167	125	−
			57	103	115	106	115	−
Avge.	33.6	− 4	Before 47	117	160	163	127	
			After 46	102	126	130	112	
			S.D. of differences 23.8	18.3	30.2	32.0	16.3	
			S.E. 2.09	5.07	8.37	8.86	4.51	
			Value of P <.01	<.01	≪.01	<.01	<.01	

* Out-patients.
† After ascorbic acid i.m.

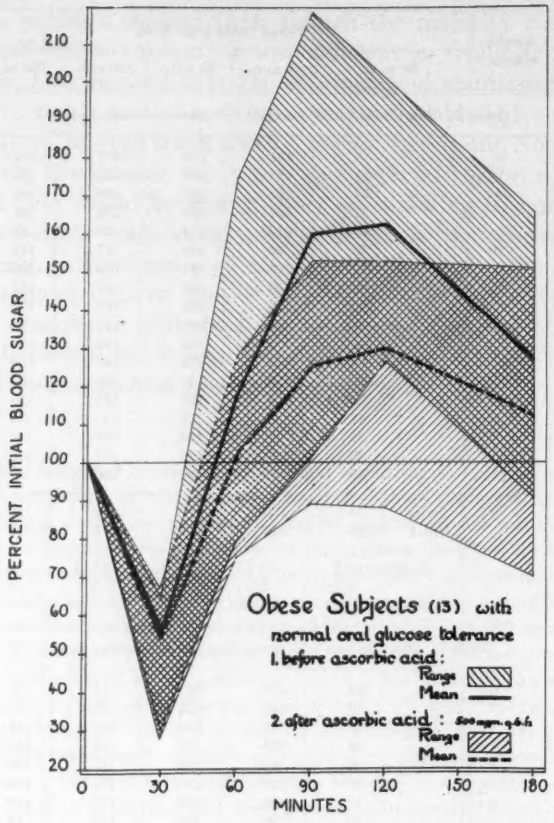

FIG. 1. The effect of 500 mg. of ascorbic acid by mouth every six hours on the insulin-glucose tolerance curve in obesity.

one or more of the individual values fell outside the normal mean ±2 S.D. These data are taken from a previous study of normal patients and are as follows (15):

Mean value	37	96	114	106	92
S.D.	7.7	24.9	21.1	17.0	10.3

The patients were arbitrarily divided into two groups according to whether the oral glucose tolerance curve was normal or not, without regard to ratio of in-patients versus out-patients. The criteria for a normal curve were a fasting blood sugar level below 120 mg. per 100 ml., a peak not higher than 170 mg., and a three-hour value which had decreased to, or near the fasting level. In the group with abnormal oral glucose tolerance curves the change after ascorbic-acid therapy was not as significant as in

the second group. No increased sensitivity to insulin was noted, as shown by the absence of hypoglycemic symptoms or lowered blood sugar levels. The patients in this first group were on the whole ten years older than the patients in the second group with normal oral glucose tolerance curves. In the latter group, a consistent intake of ascorbic acid was questionable, as

TABLE 2. THE EFFECT OF LOSS OF WEIGHT (5–15 POUNDS) ON THE INSULIN GLUCOSE TOLERANCE CURVES OF 6 OBESE PATIENTS

Patient No.	Age and sex	Weight change	% Initial blood sugar level				
			30 min.	60 min.	90 min.	120 min.	180 min.
1	30M	−12	65	127	180	180	91
			65	104	162	182	101
2	32M	−10	72	161	197	169	143
			43	129	175	166	107
3	36M	− 5	52	132	193	198	127
			43	129	169	176	94
4	44M	− 5	50	123	202	225	98
			52	120	182	205	104
5	47M	−15	74	124	178	187	135
			77	100	162	188	163
6	49M	−13	53	100	137	167	125
			55	98	193	145	140
Avge.	39.7	−10	Before 61	126	181	191	120
			After 56	115	166	177	118
			S.D. of differences 11.0	13.4	7.8	10.6	12.65
			S.E. 4.48	5.45	3.18	4.34	5.17
			Value of P <.2	<.05	<.01	<.05	<.02

the greater number of these subjects were studied on an out-patient basis. However, the results were considered significant after statistical analysis.

Tests carried out on 6 in-patients without oral ascorbic acid but having lost 5 to 15 pounds in weight did not show any marked improvement. The results are recorded in Table 2. These tests were made in order to determine whether minor fluctuations in weight might affect the response.

Routine determinations of ascorbic-acid levels in obese patients prior

to the tests did not show any evidence of hypovitaminosis. The values were all within the range of 0.6–2.5 mg. and were considered normal in our laboratory. No toxic effects from the use of large doses of ascorbic acid were observed in our study.

An *in vitro* experiment was performed in order to determine whether ascorbic acid by itself would influence the oxidation-reduction reaction used in testing for the presence of glucose. Various concentrations of ascorbic acid were added to blood samples containing either high or low concentrations of glucose. No effect on glucose values was noted. The data are shown in Table 3.

Using the same time schedule as outlined in the procedure of this study, blood sugar curves were obtained on 2 patients given 500 mg. of ascorbic acid intravenously after a fasting blood sample had been withdrawn. This

TABLE 3. THE *in vitro* EFFECT OF VARIOUS CONCENTRATIONS OF ASCORBIC ACID ON BLOOD SUGAR DETERMINATIONS

Initial blood sugar (mg./100 ml.)	Blood sugar after addition of ascorbic acid in concentrations of:		
	1 mg./100 ml.	4 mg./100 ml.	10 mg./100 ml.
90	91	91	94
320	310	310	314

test was made in order to determine whether ascorbic acid by itself would affect blood sugar determinations *in vivo*. The results were as follows:

Case A: 86, 91, 91, 84, 91, and 89 mg. per 100 ml.
Case B: 84, 85, 89, 85, 89, and 94 mg. per 100 ml.

In Cases 2 and 6, after failure with oral ascorbic acid given for a period of four weeks, it was decided to use an intramuscular preparation (500 mg. every six hours) in order to determine whether there would be a response with parenteral therapy. This had to be discontinued after three days, since the sites of injections became too painful. Only in Case 2 was there some response (Table 1). Two days later this patient's blood sugar curve was 100, 39, 130, 172, 140, and 84 mg. per 100 ml., and after one week, 100, —, 108, 159, 198, and 106 mg. per 100 ml.

The *t* test was used in the statistical analysis of this paper. The test was applied to each paired observation, before and after ascorbic-acid therapy, or before and after weight loss (Tables 1 and 2). Since our series was not large, it was decided that any value of *t* would not be considered significant if P lay between 0.05 and 0.02; was probably significant if P lay between 0.02 and 0.01; and was highly significant for P values less than 0.01.

DISCUSSION

Ascorbic acid, dehydroascorbic acid and alloxan resemble each other as to biochemical structure, and each will affect carbohydrate metabolism. Most of the experiments relating to ascorbic acid and carbohydrate metabolism have been carried out in monkeys and guinea pigs, which, like man, cannot synthesize this vitamin. Such experiments point to two possible ways whereby ascorbic acid plays a role, namely, the adrenal and the pancreas. Since stress affects the entire endocrine system, and this system is particularly rich in ascorbic acid, it would not be surprising that a relative deficiency of this substance could affect various metabolic processes. Menten and King (7) have shown that mere deprivation of ascorbic acid in the guinea pig can produce carbohydrate intolerance, even before frank scurvy is present. Since such a state is reversible to a certain extent by the administration of ascorbic acid, it seems possible that this could also be the case in the obese patient. The return of the insulin-glucose tolerance curve to normal or near normal in those of our patients who had a normal oral glucose tolerance curve seems to indicate two things. First, that the insulin-glucose tolerance test is the more sensitive one for the detection of early carbohydrate intolerance and, secondly, that an abnormal curve is reversible at this stage by the administration of large doses of ascorbic acid. Whether smaller doses would be adequate is a matter for further study. Although there may be some improvement in obese patients with marked carbohydrate intolerance, as shown by an abnormal oral glucose tolerance curve as well as an abnormal insulin-glucose tolerance curve, the results are not as significant. The possibility exists that the damage to the pancreas is irreversible or that the stress of obesity and consequent depletion of ascorbic acid has increased the release of adrenocorticotropin by the anterior pituitary, with consequent changes in carbohydrate tolerance.

The dosage of ascorbic acid chosen in our experiment (500 mg. orally every six hours), was based on studies made by Todhunter *et al.* (19). They showed that in patients with a low fasting blood level of ascorbic acid, administration of 50 to 100 mg. did not necessarily raise the blood concentration to 1.3–1.4 mg. per 100 ml.—the threshold at which renal excretion occurs. In contrast, a dose of 400 to 500 mg. not only caused a higher plasma level, but the level was sustained at a plateau above the renal threshold for more than four hours. To saturate a patient with ascorbic acid it is therefore necessary to keep the blood ascorbic acid level at, or above the renal threshold during the greater part of each day.

There is no satisfactory explanation at present for the failure of oral administration of ascorbic acid versus the success of parenteral administration, and for the clinical benefits reported following extremely large doses

versus the lack of effect when used in small doses. Case 2 illustrates the first point. Since the effect lasted for only five days after cessation of parenteral therapy, it is postulated that the obese patient does not store vitamin C and that daily large doses are necessary. There must be individual variations in absorption of vitamin C from the intestinal tract. Under certain conditions the absorption may be definitely abnormal (20), since ascorbic acid administered by subcutaneous injection may be twice as effective as when given by mouth in the protection of guinea pigs against scurvy (21).

CONCLUSIONS

1. The effect of ascorbic acid on the abnormal insulin-glucose tolerance curves in 21 obese subjects is presented. Therapy consisted of 500 mg. of ascorbic acid given orally four times a day for a minimum of three weeks.

2. The effect of such therapy was questionably significant in 8 patients with abnormal oral glucose tolerance curves. In 13 patients with normal oral glucose tolerance curves, there was a significant reversal towards normal of the insulin-glucose tolerance curve, the average readings all falling within the normal range.

3. The possible action of ascorbic acid on carbohydrate metabolism is discussed.

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INCREASED INTERMEDIN-LIKE ACTIVITY IN THE BLOOD OF PATIENTS WITH DUODENAL ULCER

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ACCORDING to Johnsson and Högberg (1), Sulman (2) and Thing (3), it is possible to recognize adrenocorticotropic hormone (corticotropin; ACTH) in the blood by means of the expanding-melanophore test. In view of the participation of the pituitary-adrenal system in the pathogenesis of peptic ulcer, it was considered of interest to study the circulating corticotropin in patients with gastric and duodenal ulcers, by means of that test. However, as shall be seen later, in the course of the work it was evident that we were dealing with an intermedin-like substance instead of corticotropin.

METHOD

The animals employed were Chilean toads, *Pleuroderma bibrioni*, of an average weight of 2.0 grams. These toads exhibit a wide range of variations of the melanophore cells under the antagonistic stimulation of light and intermedin. Contrary to the color changes observed in other frogs, the change in this toad is not in the hue of the skin, but only in lightness value. To determine the intensity of the reaction, *i.e.*, the degree of darkness, we used the orange-yellow (OY) color table of Villalobos' atlas (4). The reading of lightness value is accomplished by a comparison with the values in the column representing 8 degrees of chromaticity in this OY table. This table presents color squares of progressively increasing degrees of darkness and the squares are perforated in the centre in such a way as to permit a direct comparison with the unknown color. The toad is placed in a small flat box, the bottom of which is covered with cotton and the top of which is a plexiglass window. This box is then moved behind the 8° column of the OY color table until the exactly corresponding color square is found. We have evaluated changes in the intensity of color as follows: a change of a magnitude of 1 color square is considered doubtful; a change of 2 squares is considered positive, corresponding to 1 degree of increase in darkness on our scale. A first reading is made before the injection; a second, thirty minutes later; and a third, sixty minutes later. The toads were illuminated with a blue fluorescent light (the most effective color for inducing the retraction of the melanophores cells) twenty-four hours prior to, and throughout the test. The adaptation period to the laboratory environment was ten days, at a room temperature of about 20° C. The blood of the patients was obtained in mid-morning without previous fasting and kept for one or two days at 1° C., and in a few instances at room temperature.

Each sample of blood was injected into 4 toads in the ventral subcutis, using a tuber-

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culin syringe; 2 of the toads received 0.2 ml. of plasma, and the other 2 received 0.2 ml. of whole blood.

Another group of toads received injections, under similar conditions, with Li's or Armour's purified intermedin (R-491261) and commercial Armour corticotropin (ACTH).

Observations for this report were made on 65 patients and 5 normal subjects, divided into three groups as follows: 1) 27 controls, consisting of 22 patients with various diseases chosen at random from a hospital ward, and 5 normal individuals; care was taken not to include patients with Cushing's disease or Addison's disease, as they are known (5) to show positive results with this test, 2) 21 patients with gastric ulcer, and 3) 22 patients with duodenal ulcer. All the ulcer patients had radiologic confirmation of the lesion and were in an active phase of the disease at the time the test was performed.

RESULTS

The results are summarized in Table 1 and Figure 1. Positive reactions occurred in 14.3 per cent of the control group, in 12.2 per cent of the gastric

TABLE 1. SUMMARY OF RESULTS

(Plasma + whole blood)

	Control	Gastric ulcer	Duodenal ulcer
Number of cases	27	21	22
Number of determinations	107	82	84
Total number of toad units	6.75	7.87	34.5
Percentage of positive reactions	14.3	12.2	66.7

ulcer group, and 66.7 per cent of the duodenal ulcer group. From Figure 1 it can be seen that the intensity of the reaction was also greater in the duodenal ulcer patients.

Among the negative results, it was possible to distinguish two different types of responses. In Figure 1 there is a line that separates the definitely positive cases (black circles) from the negative cases (white circles). Of the latter, those immediately under the line actually represent doubtful reactions. The circles at the bottom, represent definitely negative reactions. The total number of definite negatives was much lower in the group of patients with duodenal ulcer (1 case, against 6 and 14 for the gastric ulcer and control groups, respectively, as shown in Table 2). Thus, if doubtful reactions are given a slightly positive value, and are added to the definitely positive responses, a still greater number of positive cases belong to the duodenal ulcer group.

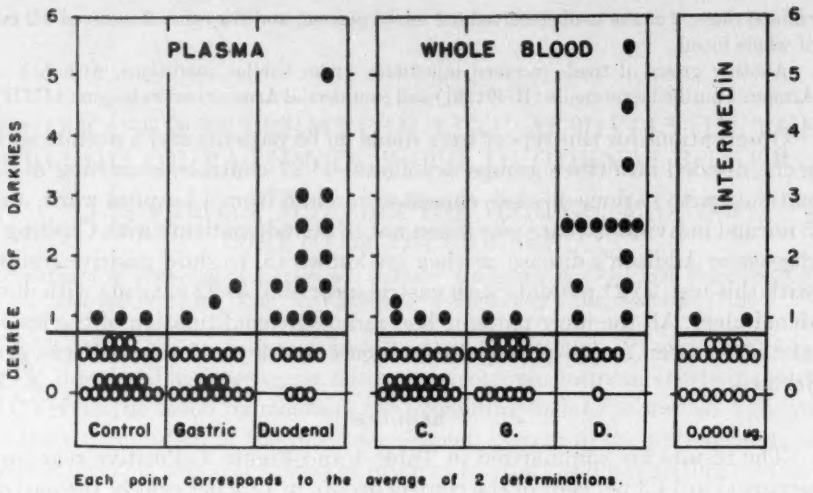

FIG. 1. Results of the expanding-melanophore test with the blood of the control, the gastric ulcer, and the duodenal ulcer patients. (Black circles = positive cases; white circles = negative cases.)

We attempted to assay by this expanding-melanophore test, the amount of Li's recently prepared purified intermedin that could be equivalent to the amount of intermedin present in the blood of our controls. We found this to be about 0.0001 µg. The values obtained with this dose are shown in the right hand column of Figure 1. Since the amount of blood admin-

TABLE 2. NEGATIVE RESULTS WITH WHOLE BLOOD

	Negative		Positive
	Absolute	Doubtful	
Duodenal	1	5	14
Gastric	6	12	2
Control	14	8	3

istered in the control group was 0.2 ml. in each determination, it is possible to assume that the physiologic concentration of the circulating intermedin could be in the neighborhood of the foregoing value, *i.e.*, 0.05 µg. per 100 ml.

Both Li's and Armour's intermedin preparations were found to contain 2.5×10^5 toad units[1] of intermedin per milligram. This result is ten times

[1] A toad unit is defined as the minimal amount of hormone capable of increasing the darkness value by 1 degree (two places of Villalobos' color table, as explained previously). Therefore, in this paper, "degree of darkness" is used as synonymous with "toad unit."

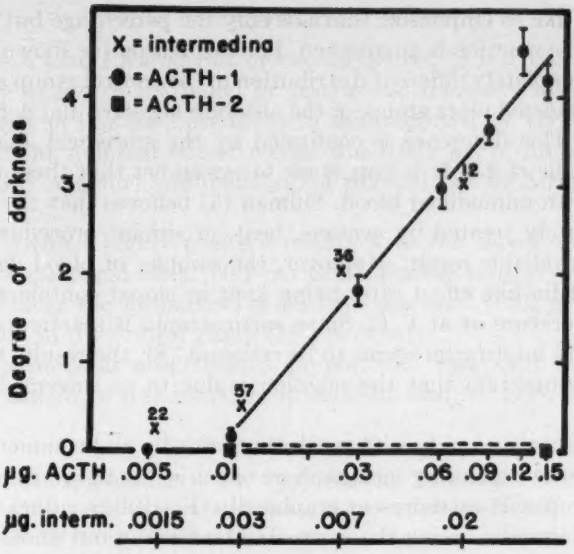

FIG. 2. Comparison of biologic assay of commercial ACTH and purified intermedin. Ordinate: degree of darkness; abscissae, dose in μg. (in logarithmic scale). ACTH-1 (black circles) refers to a commercial Armour preparation containing 3.2 U./mg., and ACTH-2 (black squares) refers to another preparation containing 1.7 U./mg. In order to superimpose the data for purified intermedin (crosses) over the standard curve, a different abscissa was employed. The dose-response curve of ACTH is based on unpublished data of Vargas, Koref and Feuereisen; each point corresponds to the average of 50 determinations. The number of determinations for each dose of intermedin is written over these averages.

lower than the one indicated in frog units by the Armour Laboratories. Armour's ACTH containing 3.2 units of corticotropin per mg. was found to have 6×10^4 toad units of intermedin per mg.; but with 1.7 units of corticotropin per mg., no intermedin activity was found within the range studied, *i.e.*, up to 0.15 μg. (Fig. 2). This means that purified intermedin, is four times more active than the ACTH preparation which contained 3.2 units per milligram.

DISCUSSION

The results obtained show that the blood level of intermedin is higher in duodenal ulcer patients during the active period of disease. Biologic assay in the control group demonstrated that the level of the circulating intermedin is around 0.05 μg. per 100 ml. of whole blood. Since the percentage of positivity and the intensity of the reaction with the blood of duodenal ulcer patients is increased fivefold, it is possible to accept that the intermedin blood level in these patients could be around 0.25 μg. per 100 ml.

We should like to emphasize that not only the percentage but also the intensity of the reaction is augmented. For this reason (as shown in Table 2) there is a completely different distribution in the control group as compared with the duodenal ulcer group, of the absolute negative and definitely positive cases. This difference is confirmed by the statistical analysis which gives a t value of 4.0. It is important to remember that these results were obtained with unmodified blood. Sulman (5) believes that the blood must be immediately treated by acetone, heat, or similar procedures, in order to obtain a reliable result. Moreover, the samples of blood demonstrated the intermedin-like effect after being kept in closed containers, either at room temperature or at 1° C. Since corticotropin is inactivated by blood enzymes and intermedin seems to be resistant (8), the results tend to support the assumption that the reaction is due to an intermedin-like substance.

Our comparative study with purified intermedin and commercial ACTH, shows that the expanding-melanophore effect is due to intermedin and not to corticotropin. If we represent graphically (Fig. 2) the values obtained for purified intermedin, using the same standard curve but another abscissa, it may be seen that the curve of intermedin overlies the standard curve. This result supports the conclusion that the effect of ACTH in this test is attributable to its contamination with intermedin. In addition, it is evident that one Armour corticotropic preparation, containing 1.7 U./mg. showed no intermedin activity, even with the highest dose of 0.15 μg. Finally, although the intermedin employed was found to have approximately fourfold melanophoric potency, it had almost no corticotropic activity (9).

All the observations mentioned here are in accordance with Li's viewpoint (6) on the separate identity of intermedin and corticotropin. It is of interest, as Sulman (5) has emphasized, that results of the expanding-melanophore test are positive whenever corticotropin is increased in the patient's blood (Sayer's test), as in Addison's disease, Cushing's disease, pregnancy and states of acute stress (10). The probable hypothesis seems to be that both hormones, corticotropin and intermedin, are secreted together under certain circumstances. Our results with peptic ulcer add another pathologic condition which sustains that inference, as this lesion is identified with an imbalance of the pituitary and adrenal, as well as an oversecretion of corticotropin during the acute stage of the disease (11). However, the hypothesis is not in line with the observations of Paris *et al.* (12), who claim that blood corticotropic activity is absent in cases of Cushing's disease, whereas sometimes intermedin activity is detected.

The difference observed between duodenal and gastric ulcers is in agreement with the concept that the former has a different patho-physiologic mechanism.

SUMMARY

The Chilean toad *Pleuroderma bibrioni* was used to test the expanding-melanophore action of the blood in gastric and duodenal ulcer patients. The sensitivity of this toad is similar to that reported by various authors for the frog: the minimal effective dose was 0.017 μg. of impure corticotropin (ACTH Armour) and 0.004 μg. of purified Li's or Armour's intermedin.

This test yielded highly positive results with the blood or plasma of patients with duodenal ulcer (66.7 per cent). On the contrary, in patients with gastric ulcer the proportion of positive reactions (12.2 per cent) was similar to that in the control group (14.3 per cent).

These experimental observations support the viewpoint that the expanding-melanophore test identifies intermedin and not corticotropin.

Acknowledgments

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SURGICAL HYPOPARATHYROIDISM PRECIPITATED BY ONSET OF THE MENOPAUSE: A CASE REPORT

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IF HYPOPARATHYROIDISM develops after thyroidectomy, it commonly becomes manifest during the first few weeks. It is most unusual for the disorder to occur almost thirty years following such an operation, as seems to be true in the case presented here.

CASE REPORT

M. O'L., a 49-year-old Irish woman, was admitted to this hospital for the first time on February 15, 1955, with the chief complaints of convulsive seizures and nervousness.

Present illness. The patient had her first convulsive seizure in June 1953, and since then several similar episodes had occurred. On the day of admission she had experienced two such seizures, one on the way to the hospital and one immediately after admission. These were described as a shaking of the arms and legs and sudden loss of consciousness not preceded by any aura. There had been no frothing at the mouth, no biting of the tongue, and neither urinary nor fecal incontinence. Following these episodes of "blacking out" the patient was weak and listless and had no recollection of the attack.

In 1954 she had been treated for an acute attack in another hospital, where the diagnoses of 1) idiopathic epilepsy (confirmed by electroencephalographic tracings) and 2) "psychic disturbance requiring further investigation" were made. At that time, treatment with diphenylhydantoin (Dilantin) was instituted.

Past history. The childhood history did not reveal any relevant facts. Thyroidectomy had been performed when she was 20 years old for the relief of Graves' disease. There had been operations for cataract in 1942, 1944 and 1947. A total hysterectomy and oophorectomy was performed in April 1953, and the pathologic diagnosis had been benign uterine polyps. She had menstruated regularly up to the time of hysterectomy. It was noted that the generalized convulsive seizures started a few weeks thereafter.

The patient has been married for nineteen years and had three healthy children 18, 16 and 9 years old, respectively. She denied the use of alcohol, tobacco or of any habit-forming drugs.

Physical examination. Physical examination revealed a well developed, slightly thin, white woman, looking somewhat older than her stated age of 49. She was in no acute distress, but seemed very tense and nervous. Her temperature was 99° F.; pulse rate, 80 per minute; respirations, 32 per minute; blood pressure, 145/95 mm. Hg; height, $64\frac{3}{4}$ inches; and weight, 113 pounds.

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She spoke in a dull monotone, with considerable repetition of words. Her facial expression was more or less fixed in a state of chronic anxiety.

Her gait was unsteady and on a broad base. However, the deep tendon reflexes were normal, and there was no Babinski sign. Chvostek's sign (type 3) was present, but Trousseau's sign could not be elicited. There were no other significant physical findings.

Laboratory data (Fig. 1). Findings of interest in the analyses made shortly after admission included the presence of small amounts of albumin in the urine, and the following concentrations of chemical constituents in the blood: urea nitrogen, 14 mg./100 cc.;

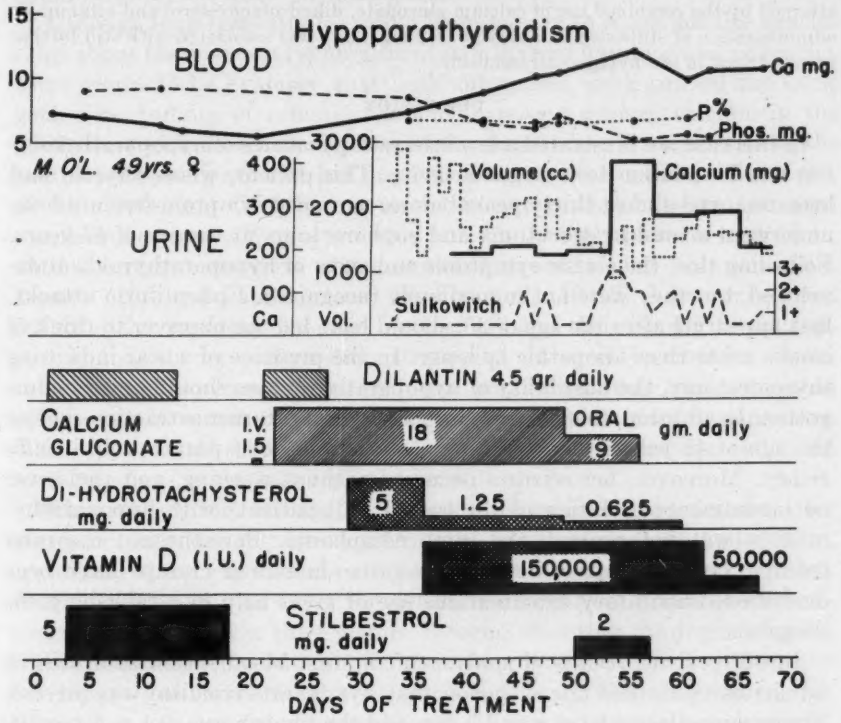

FIGURE 1

fasting sugar, 100 mg.; serum total protein, 6.7 Gm.; serum total cholesterol, 347 mg.; cholesterol esters, 178 mg.; calcium, 7.3 and 7.0 mg.; and inorganic phosphorus, 9.1 mg./100 cc.

Course (Fig. 1). The patient received 4.5 grains of Dilantin, and 5 mg. of stilbestrol daily for from one to two weeks. Then these drugs were discontinued, as the serum calcium concentration had fallen to a very low level, coincident with another seizure.

After successful treatment of the seizures with 10 per cent calcium gluconate (10 cc. intravenously), the patient was given 4 cc. (5.0 mg.) of dihydrotachysterol and 18 Gm. (12 tablets) of calcium gluconate daily by mouth. The serum calcium level rose by 1.6 mg. per 100 cc. within one week (Fig. 1). Then the dosages of calcium gluconate and

dihydrotachysterol were decreased and vitamin D, 50,000 I.U. three times daily, was begun. Shortly thereafter, stilbestrol was re-started in a reduced dosage of 2 mg. daily. The effects of these therapies were followed with the help of serum calcium, phosphorus and total protein determinations, as well as by measurements of urinary calcium and the degree of opalescence in the Sulkowitch test.

From Figure 1 it can be seen that the administration of 5 mg. of stilbestrol daily without the simultaneous use of calcium or vitamin D resulted in a further lowering of the serum calcium level. This was associated with a typical attack of parathyroid tetany. Subsequently, however, when partial control of the hypoparathyroid state had been attained by the combined use of calcium gluconate, dihydrotachysterol and vitamin D, administration of stilbestrol in a dosage of 2 mg. daily was associated with still further improvement in the hypoparathyroidism.

DISCUSSION

In this case are illustrated a few interesting features of hypoparathyroidism and its relation to estrogen activity. This patient, whose thyroid had been removed almost thirty years before, remained symptom-free until she underwent a total hysterectomy and oophorectomy at the age of 47 years. Following this, the classic symptoms and signs of hypoparathyroidism developed, but they were not immediately recognized. Epileptiform attacks, first appearing after the age of 25, should have led the observer to think of causes other than idiopathic epilepsy. In the presence of a scar indicating thyroidectomy, the possibility of hypoparathyroidism should never be forgotten. In addition, this patient had had several cataract extractions before the age of 45 years; this is almost pathognomonic of parathyroid insufficiency. Moreover, her seizures occurred without warning, and there was no incontinence or biting of the tongue. All patients with hypoparathyroidism will not present the same complaints. Paresthesias, muscular cramps, laryngeal spasms and even gastro-intestinal cramps have been described. Laboratory examinations are of great help in establishing the diagnosis.

In our patient, results of analysis of the first blood specimen drawn on admission confirmed our suspicion that hypoparathyroidism was present. The serum calcium level was 7.3 mg. and the phosphorus, 9.1 mg. per 100 cc. Kyle and co-workers (1) recently suggested that an elevation of the serum phosphorus level to between 5 and 6 mg. per 100 cc. may be the only positive laboratory finding; even the low calcium level may be absent.

Goldman and Bassett (2) and Howard and his associates (3) have recently called attention to the rapid rise in the urinary excretion of calcium when serum values have been raised above a minimum critical level through the intravenous use of calcium gluconate. The hypercalciuria observed in our patient during massive dosage with dihydrotachysterol, vitamin D and calcium gluconate may be similarly explained (2–5).

In addition to the long recognized action of parathyroid and thyroid se-

cretions on calcium and phosphorus metabolism in cases of human hypoparathyroidism, recent studies have indicated an influence of adrenal corticoids (5, 6) and estrogen (7, 8). Blohm and his associates (7) found their patient much improved during each of two pregnancies, a fact which they attributed to activity of the fetal parathyroid, but which may well have been due to the large amounts of estrogen present, particularly as improvement appeared relatively early in the pregnancies, before much activity could be expected from the fetal gland.

Although long-term studies would be necessary to reach definite conclusions about the suggested synergism of parathyroid hormone and estrogens, there seems to be evidence that both substances work toward the same goal. The findings of osteoporosis and increased urinary calcium in the menopause with its deficiency of estrogen, seem to be a contradiction of the postulated synergism and led previous investigators, such as Holtz (9), to the assumption that there is an actual antagonism between activated sterols and estrogen. This is probably true for the early phase of treatment, until a certain saturation of the depleted structures has been achieved. Zondek (10), and Landauer and Pfeiffer *et al.* (10–13), however, carried out extensive experimental work in this field and their results do not support Holtz's theory. After administration of estrogens to chickens, ducks and pigeons, they found increased formation of medullary bone associated with a rise in serum calcium concentration. That the conclusions from their experimental studies are applicable to the human being is indicated by the findings in the case reported here.

Other reasons for the onset of hypoparathyroidism three decades after thyroidectomy also justify consideration. In the patient presented here, apparently there was enough normally functioning parathyroid tissue left after thyroidectomy to cover the deficit resulting from probable inadvertent removal of some of these glands. It seems clear that the degree of parathyroid deficiency present prior to castration was sufficient to induce the development of cataracts. However, this deficiency was not great enough to result in tetany until the cessation of ovarian activity following castration. On the other hand, increasing arteriosclerotic changes in the supplying vessels could have resulted in a reduced blood supply to the parathyroids and a consequent decrease in their function.

The precise role played in hypoparathyroidism by the menopause with its lack of estrogen remains uncertain, but the relationship is sufficiently evident to warrant the use of estrogenic substances in the therapy of menopausal hypoparathyroidism. Furthermore, complete or partial removal of the ovaries in a patient who has previously undergone thyroidectomy should place the physician on guard against the possible appearance of hypoparathyroid manifestations.

SUMMARY

The case is presented of a 49-year-old woman in whom a hypoparathyroid seizure first occurred at the age of 47, twenty-eight years after a thyroidectomy, and in the same year that hysterectomy and oophorectomy were performed.

In view of the fact that the true nature of the condition was overlooked for two years, care has been taken to stress several points in diagnosis—the nature of the seizures, suggestive historical data such as the early appearance of cataracts, the presence of Chvostek's sign and a thyroidectomy scar, and the low serum calcium and high serum phosphorus levels.

In the management of this patient, an estrogenic substance aided in maintaining normal serum levels of calcium and phosphorus and induced a high urinary output of calcium.

Thus, it would seem advisable that: 1) every patient in whom hypoparathyroidism develops at the onset of the menopause receive estrogenic medication in addition to conventional measures, and 2) the surgeon who contemplates oophorectomy in a previously thyroidectomized patient recognize the fact that manifestations of latent hypoparathyroidism may first make their appearance following this procedure.

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SEXUAL AND SOMATIC PRECOCITY WITH SOME FEATURES OF CUSHING'S SYNDROME IN A 2-YEAR-OLD MALE*

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WE HAVE observed a case of somatic and sexual precocity associated with some features of Cushing's syndrome in a male child aged 20 months (the first clinical signs appeared at 11 months), caused by adrenocortical carcinoma.

CASE REPORT

E. A. was a white male aged 20 months. Until he was 11 months old he was considered normal by his family. At this age the appearance of acne on the face and buttocks, and of hair on the back and pubis was noted. The hair gradually increased and the penis grew; in one month the child gained 1 Kg. and continued to grow and gain weight rapidly. At this stage he also demonstrated exaggerated hunger and thirst. His crying was hoarse from the time of his birth. After the age of 1 year he began to have frequent erections.

There was no important previous morbid history either in the family or in the patient. He was born of a normal parturition. At 1 month he weighed 4.6 Kg. His first tooth appeared at 7 months. He walked at 10 months. Since that age he had pronounced the syllable "dá" (give me), but his vocabulary had not increased, though he understood orders, recognized common objects and ate at the table by himself like a little man.

Physical examination

He was first examined at the age of 20 months (Fig. 1). His height was 80 cm. (normal for the age in Brazil, 80 cm.), weight 15.8 Kg. (normal, 11.4 Kg.), and blood pressure 120/80.

The patient was very fat, with a "moon face," "buffalo-type" adiposity, broad shoulders, and thick, sturdy limbs. There was acne on the face and buttocks, and abundant hair on the back, pubis, and limbs. Hair was less abundant on the face, and there was none in the axillae. He had 16 teeth.

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FIG. 1. Three views of the patient, showing the large genitalia, hirsutism and the characteristic obesity.

The external genitalia had the appearance of those in early adolescence. The penis measured 3 cm., the scrotum was wrinkled, and the migratory testes had a maximum diameter of approximately 1½ cm. The development of the penis was proportionately greater than that of the testes.

Laboratory findings

Blood morphology: hemoglobin 12.8 Gm. per 100 ml.; erythrocyte count 7,250,000; leukocyte count 13,400; basophils, eosinophils, myelocytes and metamyelocytes, 0; stab, 2; segmented neutrophils, 56.5; lymphocytes, 32.5; and monocytes 9 per cent.

Blood chemistry (concentration per 100 ml.): urea nitrogen 45 mg., glucose 80 mg., creatinine 1.4 mg., calcium 12.2 mg., sodium 308 mg. (or 133.9 mEq./L), potassium 18 mg. (or 4.6 mEq./L), chloride 512 mg. (or 146.2 mEq./L), CO_2 64 vol. per cent (or 28.8 mEq./L), protein 8.3 Gm. (or 20.2 mEq./L), albumin 4.9 Gm. and globulin 3.4 Gm., with an A/G ratio of 1.4.

Urinary 17-ketosteroids: The 24-hour urinary excretion of 17-ketosteroids was 78 mg. (beta fraction, 27 per cent), 50.4 mg. and 58 mg., respectively on May 23, May 30, and June 6, 1955.

X-ray examination: skull normal; bone age 3 years; no osteoporosis. Extraperitoneal pneumography showed a tumor mass over the prolapsed left kidney.

Operation and postoperative course

Left thoracotomy was performed by resection of the tenth rib. A rounded encapsulated adrenal tumor as large as a medium-sized orange was found on the superior pole of the left kidney. The tumor was dissected and removed together with the perirenal fat.

During the dissection of the tumor, the blood pressure rose to 220/120. After removal of the tumor the blood pressure fell to 160/90. The patient awoke soon after the conclusion of the operation, in a good general condition, with a pulse of 130 and a blood pressure of 170/90.

Corticotropin and cortisone were administered before, during and after the operation.

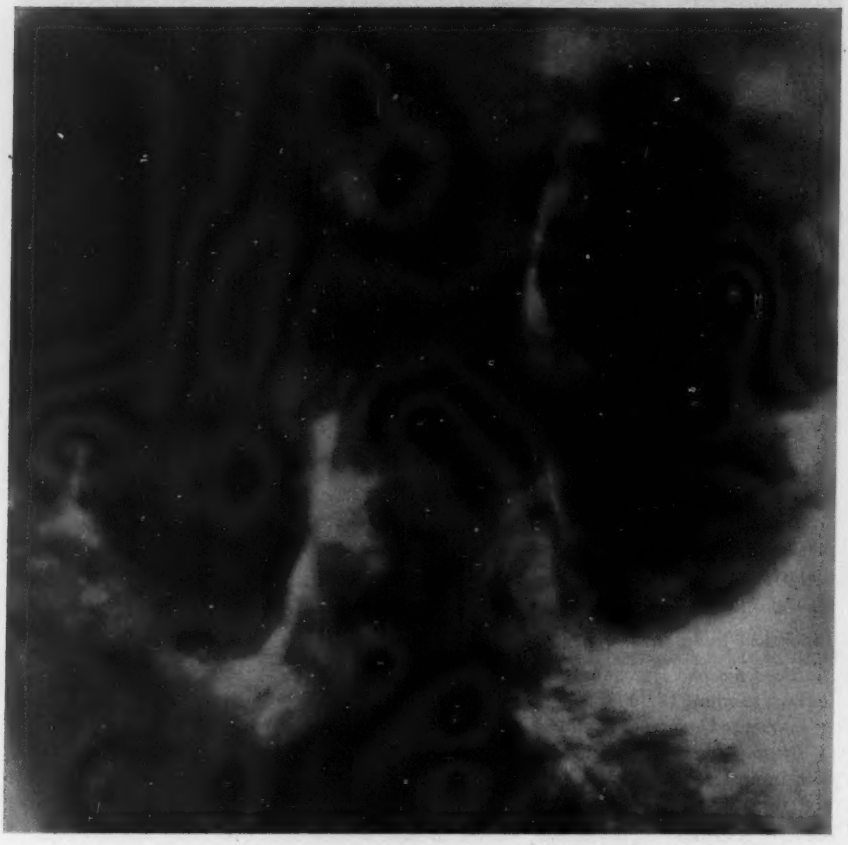

FIG. 2. Extraperitoneal pneumography. Note the large tumor of the left adrenal.

During the first twenty-four hours his condition remained satisfactory, in spite of frequent variations of the blood pressure. On the second day after the operation intense dyspnea and coughing with expectoration of mucus set in, and the patient died.

Pathology: Undifferentiated carcinoma of the adrenal cortex (Figs. 3, 4 and 5).

COMMENT

The patient showed clear signs of hyperandrogenism—enlarged penis, hirsutism, acne, advanced bone age, deepening of voice and a very high ex-

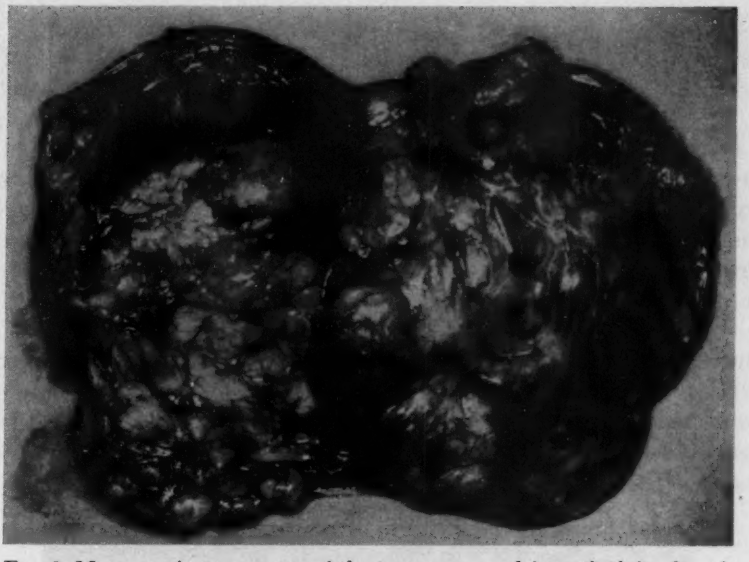

FIG. 3. Macroscopic appearance of the tumor removed from the left adrenal.

cretion of 17-ketosteroids. At the same time, he also exhibited some signs of Cushing's syndrome—typical facies, obesity of the trunk, hypertension, polycythemia, leukocytosis, absence of eosinophils, and (relative to the patient's age) lymphocytopenia, neutrophilia and an elevated blood level of urea nitrogen.

The patient did not show striae, osteoporosis, hyperlipemia or retarded growth. We suggest as an explanation the antagonistic metabolic action between androgens and glucocorticoids. (The glucocorticoid assay was rendered inaccurate by an excess of chromogens.)

Wilkins (1) states: "Reddish striae may develop but are less common than in older patients." "Glycosuria is an uncommon finding and the glucose tolerance curve may be normal" . . . "the diagnosis of Cushing's syndrome depends upon finding combinations of the many signs discussed. The patients often present a picture which is so characteristic that it is not likely to be mistaken for other conditions. There are border-line cases where the distinction between Cushing's syndrome and adrenogenital syndrome cannot be made."

Recently (1954), Bishop (2) stated: "Cushing's syndrome is commonly associated with some signs of adrenal virilism. Every case of Cushing's syndrome so far described as developing in a child under the age of ten years has been due to an adrenocortical tumor, and the majority have occurred in girls. Thus Wilkins (1950) has collected records of 26 cases under

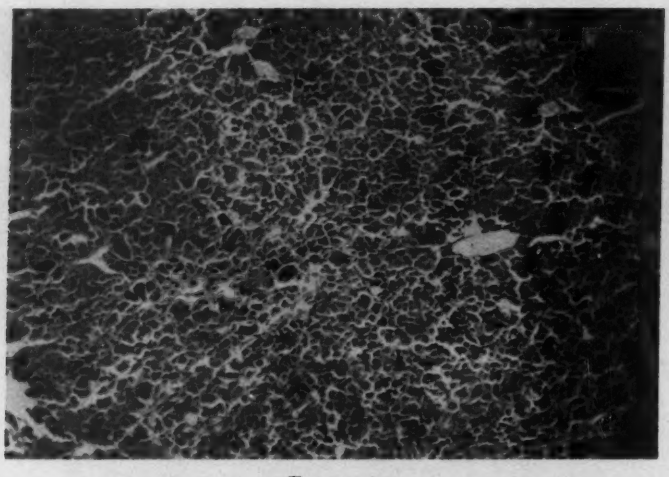

FIGURE 4

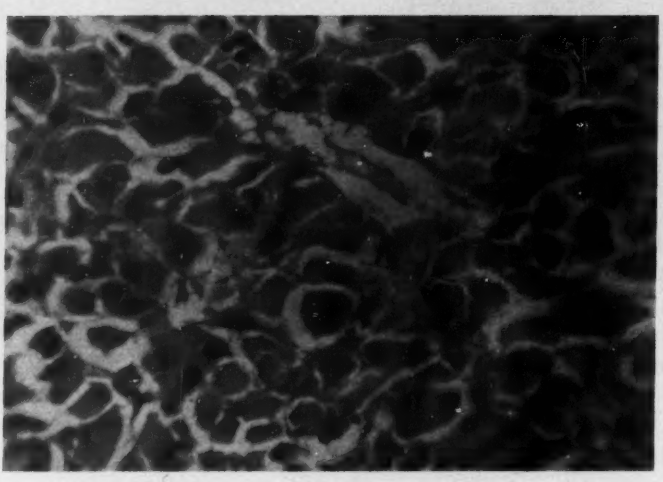

FIGURE 5

FIG. 4. Cords of epithelial cells, sometimes surrounding small blood vessels and showing an extreme grade of anaplasia.

FIG. 5. Gross enlargement, showing atypical mitosis and binucleated cells. The neoplastic cells are polyhedral and have nuclei of varying sizes.

the age of ten years, of which 22 were girls and 4 were boys. Since Wilkins' book was published 5 further cases under the age of ten years have been described. Thus, to summarize the sex incidence in these 31 patients, 24 were girls and 7 were boys."

SUMMARY

The authors present a case of sexual and somatic precocity associated with some features of Cushing's syndrome in a 20-month-old white boy. The first clinical signs appeared at 11 months of age.

The bone age was that of a 3-year-old child. The urinary excretion of 17-ketosteroids was greatly increased—78 mg. (beta fraction, 27 per cent), 50.4 mg., and 58.0 mg. per twenty-four hours on three different occasions. Blood studies showed high levels of protein, globulin and CO_2 combining power; polycythemia; leukocytosis; absence of eosinophils; low-normal levels of sodium and potassium; and high levels of urea nitrogen and creatinine.

Extraperitoneal pneumography revealed a tumor as big as a medium-sized orange in the left adrenal. The tumor was easily excised and was diagnosed histopathologically as carcinoma. Corticotropin and cortisone were administered both before and after operation. During and after operation the patient showed hypertensive crises. He died thirty-six hours post-operatively, apparently during such a crisis.

Acknowledgments

The authors wish to express their thanks for the cooperation of Dr. J. Mattoso (radiology), Drs. E. Torres and P. Dacorso (pathology), Dr. E. Magalhães and A. de Castro (laboratory).

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A CASE OF INSULIN RESISTANCE TREATED WITH CORTICOTROPIN

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THE patient with diabetes mellitus who requires more than 200 units of insulin per day is usually regarded as insulin resistant. This is a relatively rare condition, in that some 50 instances have been reported to date (1–8). The duration and the severity of the insulin resistance vary from patient to patient and from time to time in any one patient (1, 8). Nevertheless, insulin resistance is a condition which is self limited (4, 8).

The present report deals with a patient in whom there developed a resistance to insulin which was apparently influenced favorably by the administration of corticotropin.

CASE REPORT

A 62-year-old Israeli-born Jewish man was admitted for uncontrolled diabetes mellitus and insulin resistance.

His parents had died from complications due to diabetes. One brother and one sister were reported to have had mild diabetes. Another brother and sister were healthy.

Diabetes mellitus had been diagnosed in this patient twenty-five years before. It had been controlled by diet alone and only occasionally had insulin been necessary. In the year preceding entry the patient had required an average of 48 units of protamine zinc insulin (PZI) and regular insulin per day.

In December 1952, his clinical condition became more severe and he lost some 15 kilograms in weight. He was admitted to a medical ward in Haifa in April 1953.[1] During his six weeks' stay there, his blood sugar level fluctuated between 300 and 400 mg. per 100 ml. The output of urine was about 3,500 ml. per day with 5–6 per cent sugar. A dosage of 100 units of PZI and 200 units of regular insulin was insufficient to maintain aglycosuria. An insulin tolerance test showed a drop in the blood sugar level from 380 mg. to 310 mg. per 100 ml. in fifteen minutes. An electrocardiogram obtained during the period of hospitalization was found to be normal. The only positive finding of note was hypertrophy of the prostate with a residual urine of 150–250 ml. He left the hospital on a maintenance dosage of 360 units of insulin daily.

In July, 1953, he was admitted to another Haifa hospital with a blood sugar level of 410 mg. per 100 ml., urea 52 mg. per 100 ml., CO_2 combining power 37.5 volumes per

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[1] We are indebted to Dr. Heichman, Chief of the Medical Department, Government Hospital "Rambam," Haifa, and to Dr. Rotter, Haifa, for permission to use part of their examination notes, and for referring the patient to our Department.

cent, and a urinary sugar excretion of 170 Gm. per day. Treatment at this time consisted of 310 units of PZI, 300 units of regular insulin, and a diet containing 100 Gm. of carbohydrate, 70 Gm. of protein and 50 Gm. of fat. When the diet was increased to 260 Gm. of carbohydrate, his blood sugar level, which had fallen to about 150 mg., rose to 400 mg. per 100 ml. He was given potassium chloride and antihistamines because of the appearance of urticaria at the site of the injection. He was again discharged.

After a short period at home, he was readmitted in diabetic coma in spite of receiving a daily dose of 700 units of insulin. He was given 1,050 units of regular insulin to bring him out of the coma. Subsequently he was maintained on 700 units of insulin (300 units of PZI, and 400 units of regular insulin divided into eight daily doses of 50 units each). At this time, his diet consisted of 140 Gm. of carbohydrate, 70 Gm. of protein and 50 Gm. of fat. His blood sugar level fluctuated around 390 mg. per 100 ml., urinary sugar excretion was 70 Gm. in twenty-four hours, and body weight was 55 Kg. The electrocardiogram showed depression of the ST segment and inversion of the T waves in all the standard leads.

The patient was then transferred to our hospital for further treatment. He complained of excessive fatigue, pain in the legs, marked loss of weight, and the necessity for using excessive amounts of insulin in order to control his metabolic disturbance. He was listless, restless and markedly undernourished. Arterial pulses in both feet were diminished. The residual urine was 90 ml. An oscillogram showed absence of pulsation in both feet and diminished oscillations in both legs (maximum, left leg, 40; right leg, 20). The prostate was moderately enlarged. The eyegrounds showed narrowing of the arteries, hyper-reflexia, occasional arteriovenous compression and a few white spots. The basal metabolic rate was +10 per cent. The maximum urea clearance (Van Slyke method) was 145 per cent; the standard clearance was 160 per cent. Roentgen examination of the chest revealed a slight enlargement of the left ventricle. An intravenous pyelogram did not reveal any abnormal findings, nor were any calcified deposits seen in the pancreatic or renal areas. The skull roentgenogram was normal. The gallbladder did not fill with contrast material. The electrocardiogram showed changes compatible with healed myocardial infarction.

Laboratory examinations showed the following blood levels: urea, 49 mg. per 100 ml.; cholesterol, 288 mg. per 100 ml.; alkaline phosphatase, 4.3 units; acid phosphatase, 0.45 units; phosphorus, 3.9 mg. per 100 ml.; thymol turbidity, 2.7 units; Weltman coagulation band, No. 6; diastase, 20 units; total protein, 6.75 Gm. per cent; albumin 4.45 Gm. per cent; globulin 2.30 Gm. per cent; CO_2 combining power, 65 volumes per cent; sodium, 139 mEq./L; and potassium, 4.5 mEq./L. Wassermann and Kahn reactions were negative. During the course of his stay in the hospital, there was no essential change in these findings.

The patient's course in the hospital is charted in Figure 1. He was maintained originally on a diet of carbohydrate, 150 Gm.; fat, 90 Gm.; and protein, 120 Gm.—(Diet I). Later he received carbohydrate, 230 Gm.; fat, 90 Gm.; and protein, 120 Gm.—(Diet II). Varying amounts of insulin were given, up to nearly 600 units. The insulin, when injected intravenously in repeated doses, reduced the blood sugar level, but never to near-normal values. Although the intravenous route was somewhat more effective than the subcutaneous route, intravenous administration was abandoned because the patient repeatedly complained about anginal pain after such injections.

Because of the possibility that the insulin resistance might be caused by an insulin antibody, and since corticotropin had been used successfully once before in a similar case (4), we decided to try corticotropin therapy.

For three days the corticotropin was given intravenously, but afterwards as a gel in doses of 100 mg. per day. It was found that the dose of insulin necessary to control the blood sugar level and urinary sugar excretion during corticotropin therapy could be reduced progressively from 600 units to about 50 units per day. Corticotropin was discontinued after twenty-five days, when it appeared that the patient was no longer resistant to the action of insulin. As illustrated in Figure 1, the administration of cortico-

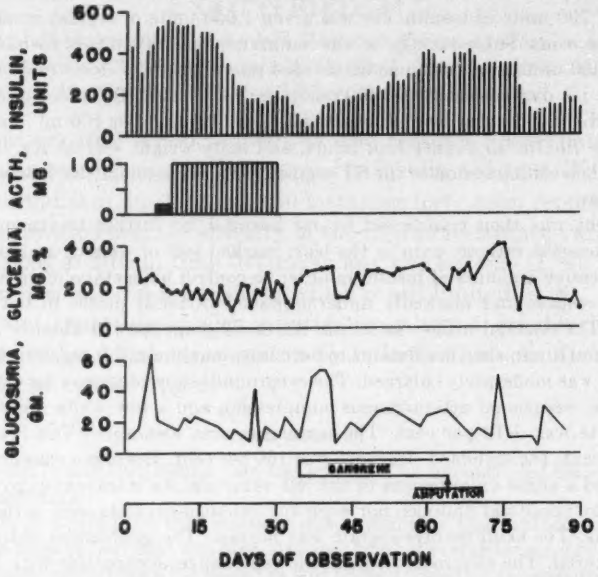

FIG. 1. The patient's daily insulin requirements, fasting blood sugar levels and glycosuria. (The solid black rectangle represents corticotropin given by intravenous drip. The rectangle of vertical lines represents corticotropin given in the form of ACTH gel.) Note the drop in daily insulin requirements during corticotropin therapy and the simultaneous decrease in the blood sugar level and the glycosuria. The onset of gangrene of the leg was associated with an increase in the insulin requirement, which dropped only after amputation.

tropin was associated with a reduction in the quantity of sugar excreted and a steady gain in body weight which was not due to edema. He felt very much better, became cheerful, showed a marked increase in appetite, and became very active in contrast to his previous almost bedridden state.

Three days after corticotropin had been discontinued, he had a sudden attack of pain in his left leg which was due to an arterial occlusion. Gangrene of the left foot developed within the next four to five days. During this period, the patient became resistant again to insulin and his condition deteriorated. The pain increased daily, appetite diminished, and the initial ischemia spread (in the form of a triangle) from the external aspect of the ankle to the tips of the large and second toes. Later frank gangrene appeared, with phlyctenular and necrotic areas. The insulin requirement rose to 400 units daily but even this amount could not control the metabolic disturbance. After heparin therapy

and repeated paravertebral blocks had failed to prevent the spread of gangrene, the affected limb was amputated at the level of the lower third of the thigh.

The postoperative period was complicated by a urinary infection, which was treated with antibiotics. After this period the amputation stump healed. The patient's insulin requirement fluctuated between 350–400 units.

Instead of starting a fresh course of corticotropin, a trial with a new type of insulin was made. The patient got the Danish "Novo Insulin Semilente" and "Lente,"[2] and a satisfactory reduction from 400 units to 150 and 130 units was achieved. The patient was discharged on a regimen of 130 units of daily subcutaneous Novo Insulin Semilente. His general state was good.

TABLE 1. TEST FOR INSULIN NEUTRALIZING ACTIVITY

After a ten-minute period of incubation at room temperature, 0.5 ml. of a solution containing insulin plus saline or serum was injected intraperitoneally into mice weighing from 16 to 22 Gm.

Date	No. of animals in each group	Insulin* dosage (milli-units)	Per cent with hypoglycemic response to insulin		
			Saline	Normal serum	Patient's serum
Sept. 27, '53	10	40	100	100	0
Oct. 21, '53	5	40	100	20	100
Nov. 15, '53	4	50	75	50	0
Nov. 30, '53	5	70	80	60	0
Jan. 1, '54	5	200	100	100	100

* Insulin dosage was changed according to the results of the preliminary test for hypoglycemic response.

During the first part of his stay in the hospital special examinations were performed in an attempt to elucidate the mechanism of the insulin resistance.

In the passive transference test (Prausnitz-Kuestner method (6, 12)) the patient's serum sensitized the skin of a normal control subject to insulin. Wheals appeared following the injection of 0.1 ml. of the patient's serum into the skin and, twenty-four hours later, of 0.02 ml. of various insulin dilutions up to 1:16.

In another set of observations, the patient's serum was tested for its ability to protect fasted mice against the hypoglycemic effects of insulin (Table 1). In these assays the amount of insulin needed to induce hypoglycemia was determined prior to each test on fasted control mice. New mice were used for successive determinations. As summarized in Table 1, at first

[2] We gratefully acknowledge the provision of a sufficient quantity of "Novo Insulin Semilente" and "Lente" by Novo Laboratories, Copenhagen, Denmark.

the patient's serum decreased the hypoglycemic effects of insulin as compared with the serum of a normal subject, which afforded no such protection. This protective or anti-insulin effect of the patient's serum was shown by the fact that in the mice receiving the mixture of insulin and serum neither paralysis nor convulsions developed, nor did any of the animals die. However, in the control mice which received a mixture of insulin and normal serum or saline, these changes did develop. After administration of corticotropin the anti-insulin effect disappeared, but recurred again in association with the reappearance of insulin resistance.

In an attempt to clarify the mechanism of the insulin resistance and with the idea that it might be due to a specific enzyme, insulinase (9), we treated the serum in various ways, such as with 0.0005 M copper sulfate, 0.001 M iodoacetate, dialysis for three hours against distilled water, or heating at 65° C. for half an hour; but equivocal results were obtained.

When the patient's serum was tested for its protective qualities against the hypoglycemic effects of insulin on mice during the last stage of renewed insulin sensitivity, it was found that it had lost its anti-insulin effect (Table 1).

DISCUSSION

The fact that the intravenous administration of several hundred units of insulin was required to regulate the glycosuria indicates that the patient had a resistance to insulin rather than some disturbance in the absorption of insulin from the site of injection (12).

Many have tried to associate the state of true insulin resistance with some concomitant disease. Although it is generally acknowledged that infections, malignant growths, liver disease, pancreatic diseases, thyrotoxicosis, and perhaps hemochromatosis may be associated with an increase in the requirements for insulin, nevertheless, there are many instances in which insulin resistance cannot be associated with any coexisting disease. The patient reported here suffered from hypertrophy of the prostate, arteriosclerotic cardiovascular disease, generalized atheromatosis, and gallbladder disease. These conditions, however, were clinically silent and remained so during the pronounced fluctuations in the insulin resistance. Moreover, the occurrence of such conditions together with diabetes in the same patient and for many years is much more frequent than true insulin resistance (8) and should, therefore, be considered only as a coincidence in this patient.

Some authors believe that insulin resistance may result from the development of an insulin antibody (6, 8, 11, 12, 16) which differs from the one causing skin sensitization (6, 12). The two types of antibody are not always found together (1, 16). One case has been reported in which the gamma globulins isolated from the plasma of an insulin-resistant patient

protected rats against the hypoglycemic effect of insulin (15). Our patient showed local skin sensitivity (urticaria) and a positive reaction with the Prausnitz-Kuestner passive transference test; the intracutaneous reaction to insulin was positive. The diabetes was partially regulated with large amounts of insulin, and this amount could be somewhat reduced when the intravenous route was used. This fact suggests the possibility that the resistance was due to some antibody mechanism, in that the antibody was bound and could be neutralized by the excess of insulin which could therefore exercise its effect on the diabetes (6).

The rationale for the use of corticotropin is based on the possibility that antibody formation or antibody union might be eliminated thereby. The increase in weight which followed the administration of corticotropin may have been partly due to the increased appetite stimulated by this treatment, but must be attributed mainly to the regulation of the metabolic derangement, since the insulin requirement diminished progressively during the period of corticotropin administration.

Corticotropin was discontinued when it appeared that maximal benefit had been obtained and that further administration might have a reverse effect, in that it could delay further improvement and induce a "steroid diabetes" (13, 14). This assumption was supported by the further reduction in insulin requirement which followed the cessation of corticotropin therapy (Fig. 1).

The reappearance of insulin resistance when gangrene set in should be noted. Once the complication was removed, and without specific treatment for the insulin resistance, this refractoriness disappeared. It may be that it was a short period of self-limited resistance, which disappeared when the toxic gangrenous condition and the shock of the operation were overcome. It may also be that the choice of another variety of insulin contributed to the return of the carbohydrate metabolism toward normal levels. The final improvement with a different type of insulin, and the disappearance of the anti-insulin factor of the serum, can probably be attributed to the improvement in the patient's condition after the amputation.

SUMMARY

Insulin resistance developed in a case of severe diabetes in a 62-year-old man. The diabetes could not be regulated by regular insulin in dosages up to 600 units daily. Administration of corticotropin effected a dramatic improvement in his general condition within a short period and permitted his daily insulin dose to be reduced to 80 or 100 units.

Later, the development of gangrene in one leg caused a temporary exacerbation in the patient's disease; but amputation, together with the use of a new form of insulin, restored him again to the state of well-being which he had achieved before the onset of gangrene.

The marked therapeutic effect of corticotropin in this case supports the assumption that the insulin resistance was due to an antigen-antibody reaction.

The presence of an anti-insulin factor in the patient's serum could be demonstrated on mice, simultaneously with the presence of resistance to insulin. With the disappearance of the resistance, the insulin-neutralizing factor in the serum also disappeared.

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Editorial

A MATTER OF GREAT IMPORTANCE TO MEDICINE—THE PLIGHT OF THE ARMED FORCES MEDICAL LIBRARY

THE Armed Forces Medical Library, the finest collection of medical literature in the world, is sorely in need of a new home and an adequate operating budget. Harrassed by insufficient provision for proper care, it is constantly threatened by loss from fire, and has actually undergone damage from exposure and improper storage. This irreplaceable treasure is vitally related to the health and progress of the nation. Not only does it contain an unequalled store of material essential to medical research, teaching and practice, but it maintains an active and invaluable service in making this material available to civilian as well as military members of all the health professions. To all medical workers, the services that such a great library can provide are indispensable. Knowledge of new experimental and clinical data must be available at the earliest possible moment. The library organizes this scattered mass of literature for distribution to those seeking information. Access to such material not only stimulates interest in further investigation, but the up-to-date knowledge of prior discoveries helps in the wise planning of new projects and prevents possible wasteful spending of research grants.

The library was created in 1836 by Dr. Joseph Lovell, the first Surgeon General of the Army. During Colonel John Shaw Billings' tenure as Surgeon General (1865–1895), it was expanded, and in 1887 was installed in the present structure. Here it houses over 650,000 books, and is adding to them at the rate of over 25,000 volumes a year. About 100,000 journals and monographic pieces are received annually. All this requires about a mile of linear shelf footage per year. As an example of the various services, some 8,000 orders are filled every month through the photoduplication department of its loan section. Its two major publications are the monthly Current List of Medical Literature[1] and the annual Armed Forces Medical Library Catalog. Occasional bibliographies are also published, such as the massive one soon to appear on the Chemotherapy of Cancer.

This vast collection, which is of incalculable value, is now maintained in a building constructed in 1887 and condemned thirty years ago as in-

[1] This editor can pay unequivocal tribute to the worth of this aspect of the AFML services. The Current List of Medical Literature is the only comprehensive index in this country which is up to date, and as such is invaluable to an editor. During the past year, economy in the use of library funds has become evident with visible forcefulness. Formerly, CLML was printed in ordinary readable type. In order to cover the same amount of work on a restricted budget, the index is now printed in type so small as to be barely readable without magnification.

adequate for library services; it is so antiquated that its iron columns have actually buckled. In 1942, the History of Medicine Division was moved to Cleveland where, in 1952, it was necessary to rent additional storage space in a warehouse. Beginning in 1944, several other divisions were moved back and forth among various buildings in Washington, until such changes could be made in the old main building as to enable the gathering of this material once again under one roof. Now, within the next two years, the library will again be faced with the problem of space, and it is hoped that by that time a new home will be assured before bursting bookshelves once more force costly and discouraging measures of desperation.

The nation has every reason to be grateful to the Surgeons General of the Army for fostering such a superb contribution to medicine. The character of the library has changed over the years, and it has long ago grown beyond the limits of military use, until it is now a treasure that is national in scope. Thus there is need to give this unique institution a national status in action as well as in spirit, with an appropriate administrative organization, an effective building, and a budget which will be adequate for performing pertinent functions.

The library is now at the crossroads so far as adequate financial support is concerned. At present it is operated under the Department of Defense and its expenses are covered in 18 different categories. Since 1918, repeated attempts have been made to obtain a new building. The latest attempt seemed promising and funds were actually appropriated by Congress last summer for architect's plans. However, these plans came to a halt when the Department of Defense decided that provision for construction could not be carried in the defense budget for the fiscal year 1957, now before Congress. It is understandable that, even in peace time, the funds for upkeep of the library cannot compete with the urgent needs of the military to maintain an effective fighting force. Thus, it seems necessary to seek another solution. In view of the large sums which Congress will be in the process of appropriating year after year for medical research, it would seem reasonable to allot the small fraction required once in a generation for the agency which provides fundamental tools for the achievement of this objective. Such a bill, sponsored by the Chairman of the Committee on Labor and Public Welfare and his colleague, is now undergoing hearings in Congress.

This is an affirmative, constructive matter which requires the cooperation of all concerned. The circumstances are particularly favorable at present. All who are interested in the medical welfare of the nation should become fully informed[2] about the need to obtain adequate support for this priceless library before further neglect takes its toll.

[2] Additional information can be procured from Colonel Frank B. Rogers (MC), Director, Armed Forces Medical Library, Washington 25, D. C.

Letters to the Editor

CHROMATIN TEST IN KLINEFELTER'S SYNDROME

To the Editor:

The oral smear technique (1) for determining genetic sex has offered additional information in the evaluation of patients with atypical sexual development.

Recently, a 19-year-old "male" was admitted for psychiatric appraisal. On physical examination, the testes were scrotal and small, but the penis was normal. On rectal examination the prostate was palpable. Panendoscopic examination revealed a normal anterior and prostatic urethra; the utricle and ejaculatory ducts were normal. The pubic hair distribution was that of a female and there was bilateral gynecomastia. A specimen of seminal plasma was devoid of spermatozoa. Urinary 17-ketosteroids were normal and gonadotropins were elevated as in a castrate. On surgical exposure the testes were very small but definite epididymal bodies were present. Bilateral testicular biopsies showed tubular sclerosis with normal Leydig cells. These findings were compatible with the Klinefelter's syndrome. The oral smear was that of a genetic female.

The finding of a female type of oral smear stimulated us to examine 4 patients who had previously been studied and diagnosed as having Klinefelter's syndrome. Their oral smears were also those of genetic females.

It is now generally recognized that a high percentage of patients with the syndrome of "ovarian agenesis" are genetic males (2). The finding of female chromatin in "males" offers a new concept for further studies of the histopathogenesis of Klinefelter's syndrome.

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LACK OF SECRETION OF 17-HYDROXYCORTICOSTEROIDS IN A PREGNANT WOMAN WITH ADDISON'S DISEASE

TO THE EDITOR:

Adrenal hypertrophy, remission of rheumatism, and aggravation of diabetes during pregnancy are well known facts. An increased production of "corticoids" during pregnancy would seem to be assured if one considers the rise of free glucocorticoids (1), free (2–3) and conjugated (3) reducing lipids, formaldehydogenic substances (4), and DNPH[1] corticoids (5) in the urine, as well as the rise of free 17-hydroxycorticoids in the blood (6–7). On the other hand, experimental work on pregnant adrenalectomized animals (8–9), the possibility of being able to discontinue adrenal steroid therapy in pregnant addisonian women (10), and the findings of Knowlton and Jailer (11–12) concerning these patients, indicate a "cortisone-like" activity of the placenta. Johnson (13) was able to detect some free glucocorticoid-like substances in the normal placenta, but such a view is not supported by Venning (14); de Courcy found traces of cortisone and hydrocortisone in the placenta (15).

We had the opportunity to study the case of an addisonian pregnant woman and concluded that the placenta does not secrete cortisone or hydrocortisone.

CASE REPORT

Mrs. Bou. had been a typical addisonian for three years when she became pregnant. The diagnosis of Addison's disease was supported by asthenia, previous tuberculosis, hypotension, hypoglycemia, results of hormonal assays and the occurrence of an acute crisis whenever the compensating treatment was interrupted. She was receiving 25 mg. of cortisone acetate orally per twenty-four hours, and 5 mg. of desoxycorticosterone acetate intramuscularly every other day. Her general condition was so satisfactory that the treatment was reduced to 12.5 mg. of cortisone per day. It was even omitted for a fortnight, under medical care, without any trouble, The delivery of a male child weighing 3,080 grams took place fifteen days later than expected. At that time rather large doses of cortisone and desoxycorticosterone were given.

Table 1 shows that the excretion of pregnandiol and that of estrogens were practically normal or slightly lower than normal. On the other hand, urinary 17-hydroxycorticosteroids (Porter-Silber chromogens) disappeared when treatment with cortisone was interrupted.

Paper chromatography of the extract in week 31 (Table 1) showed the absence of cortisone and hydrocortisone and yielded only 50 μg. of tetra-

[1] Producing a color with dinitrophenylhydrazine.

TABLE 1. EXCRETION OF STEROIDS IN A WOMAN WITH ADDISON'S DISEASE, BEFORE AND AFTER DELIVERY OF A MALE CHILD

Week*	26	31	33	36	37	38	1	3	7
Treatment† (mg./day)	*12.5 E*	*0*	*0*	*12.5 E*	*12.5 E*	*12.5 E*	*75.0 E*	*75.0 E*	*25.0 F*
Urinary vol. (cc./day)	1,400	630	950	1,370	1,140	1,000	730	2,800	1,500
Creatinine (Gm./day)	0.86	1.00	0.80	0.87	0.74	0.93	0.65	1.06	0.65
Pregnandiol‡ (mg./day)	49.5	41.0	—	64.0	49.3	66.6	2.8	2.1	—
Phenolsteroids§ (estrogens) (mg./day)	3.73	3.45	3.84	5.71	5.13	6.89	0.070	0	—
17-Ketosteroids‖ (mg./day)	2.8	3.4	1.2	7.7	5.5	7.6	—	2.5	1.2
17-Hydroxycorticoids¶ (mg./day)	2.8	0	0	9.0	—	—	—	16.8	4.2
DNPH corticoids# (mg./day)	14.2	15.0	18.0	29.2	—	—	—	24.7	7.2

* From the first day of the last menstrual period (weeks 26–38) and after delivery (weeks 1–7).
† E = Cortisone. F = Hydrocortisone.
‡ "Pregnandiol-like" (Venning).
§ Enzymatic hydrolysis; Kober's reaction.
‖ Hot-acid hydrolysis.
¶ Porter-Silber chromogens, Reddy modified technic.
# Enzymatic hydrolysis; Gornall-MacDonald reaction.

TABLE 2. CORTICOTROPIN (ACTH) TESTS, BEFORE AND AFTER DELIVERY.
(I = 24 HRS. BEFORE ACTH. II = 24 HRS. AFTER BEGINNING OF 8-HOUR INFUSION OF ACTH, 25 MG.)

Week*	26		31		3	
Treatment† (mg./day)	*12.5 E*		*0*		*75.0 E*	
CORTICOTROPIN (ACTH)	I	II	I	II	I	II
Urinary vol. (cc./day)	1,400	1,000	630	1,000	2,800	2,470
Creatinine (Gm./day)	0.86	0.91	1.00	0.80	1.06	0.99
Pregnandiol‡ (mg./day)	49.5	—	41.0	33.0	2.1	4.3
Phenolsteroids§ (estrogens) (mg./day)	3.73	2.27	3.45	4.14	0	0
17-Ketosteroids‖ (mg./day)	2.8	1.8	3.4	3.5	2.5	2.5
17-Hydroxycorticoid¶ (mg./day)	2.8	1.6	0	0	16.8	18.7
DNPH corticoids# (mg./day)	14.2	13.8	15.0	15.0	24.7	21.6
Blood eosinophils (per cu. mm.)			140	Hours 4 = 130 6 = 140 24 = 160	110	Hours 4 = — 6 = 160 24 = 120

* From the first day of the last menstrual period (weeks 26–38) and after delivery (weeks 1–7).
† E = Cortisone. F = Hydrocortisone.
‡ "Pregnandiol-like" (Venning).
§ Enzymatic hydrolysis; Kober's reaction.
‖ Hot-acid hydrolysis.
¶ Porter-Silber chromogens, Reddy modified technic.
# Enzymatic hydrolysis; Gornall-MacDonald reaction.

hydrocortisone and 50 μg. of tetrahydro-hydrocortisone per day after two days without any treatment.[2]

DNPH corticoids were above normal values and approximately those of normal pregnant women.[3]

17-Ketosteroids, assayed with the Zimmermann reaction, were elevated, as is usual during pregnancy, owing to an increased amount of 20-ketosteroids.

The rate of secreted steroids and the eosinophil count during and after the course of pregnancy were not modified by administration of 25 mg. of corticotropin (ACTH) intravenously over an eight-hour period (Table 2).

After delivery, all steroid levels returned to the values which are normal for an addisonian woman treated with hydrocortisone.

CONCLUSION

This case history shows that the placenta does not secrete 17-hydroxycorticosteroids. However, as in normal pregnant women, it produces progestative and estrogenic hormones typical of pregnancy, and DNPH "corticoids." These secretions are not influenced, as in normal pregnant women, by administration of corticotropin.

We suggest:

1. The placenta does not produce cortisone and hydrocortisone, as represented by 17-hydroxycorticoids in the urine.

2. Clinical improvement in addisonian pregnant women and adrenalectomized animals seems rather to be due to the atypical corticoids produced by the placenta.

3. In normal pregnant women, on the other hand, there is a rise in the level of free 17-hydroxycorticoids in blood (6–7) and in urine (3). The conjugated tetrahydrogenated 17-hydroxycorticoids are not increased (3–16). This discrepancy may be the result of insufficient conjugation in the liver, due to the very high level of the hormones secreted in pregnancy.

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[2] Hydrolysis with β-glucuronidase and sulfatase of gastric secretion of Helix pomatia; $CHCl_3$ extraction; benzene-water partition; Whatman No. 2, 35° C. 3 hr. systems: toluene 100-methanol 50-water 50, and benzene 100-methanol 50-water 50; detection and semiquantitative assay of ketols (triphenyltetrazolium) and of Δ^4-3-ketosteroids (fluorescence in ultraviolet rays after heating in an alkaline medium).

[3] Our chromatograms have shown an unidentified α-ketolic steroid, less polar than cortisone and hydrocortisone metabolites and failing to show the Porter-Silber reaction.

Acknowledgment

We wish to acknowledge the help of Dr. J. Louchart for the translation.

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IMPROVED AUTOMATIC FEED IN THE LAKSHMANAN-LIEBERMAN APPARATUS FOR GRADIENT ELUTION CHROMATOGRAPHY

TO THE EDITOR:

In applying gradient elution chromatography to our studies of urinary ketosteroids, we found the Lakshmanan-Lieberman apparatus (1) quite helpful. Despite its many good automatic features, however, this method still did not obviate the necessity for frequent adjustment of the drop feed. In other words, a technician had to be in frequent attendance upon the apparatus to make it work at maximum efficiency.

We have been able to save both time and annoyance by eliminating the drip method of feeding the column. Figure 1 illustrates this new adaptation. These changes concern only that part of the apparatus between the petcock below the mixing chamber and the alumina column.

Just below the petcock we inserted a ground-glass joint (A), to which

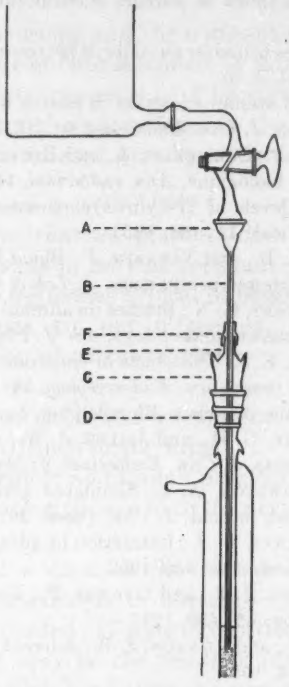

FIG. 1. (A) Ball joint, 18/7 mm. (B) Capillary tubing (size of bore not critical), length 12 in. (C) Ground-glass joint (female) 19/38 mm. (D) Ground-glass joint (male) 19/38 mm. (E) Projecting cuff into which capillary fits. (F) Rubber sleeve (must fit both cuff and capillary).

is attached a capillary tube (B). The alumina column is supplied at its upper end with a ground-glass joint (C) rather than with the rubber stopper used in the apparatus as originally described. The upper part of the stopper, the male part (D), is designed with a projecting cuff (E) which receives a piece of rubber tubing (F) that fits the capillary tube snugly and makes the whole apparatus airtight. This cuff serves the purpose of allowing adjustment of the capillary tubing so that it is at all times kept just above the top of the alumina column. With this simple, airtight design,

the level of fluid above the column is at a minimum and is constant for any given air pressure. It will be noted that the air space above the alumina column has been reduced to a fraction of that present with the conventional Lakshmanan-Lieberman apparatus. This results in a lower level of fluid above the column and a decrease in the variation of height of such fluid. If desired, this effect can be further augmented by the use of a very thick-walled capillary tube.

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The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 7-8-9, 1956.*

The Committee on Local Arrangements is Dr. Norris J. Heckel as Chairman with Drs. M. Edward Davis, Sam Taylor, J. P. Greenhill, and Harley E. Cluxton as members of the Committee.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

HENRY H. TURNER, M.D.
Secretary-Treasurer,
1200 North Walker Street,
Oklahoma City, Oklahoma.

(For program, *see* April issue of the Journal)

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.

3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE EIGHTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY; THE UNIVERSITY OF TEXAS POSTGRADUATE SCHOOL OF MEDICINE; AND THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, HOUSTON, TEXAS

October 22-27, 1956

The faculty will consist of twenty-two eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program and registration, write to *Dr. J. B. Trunnell, M. D. Anderson Hospital, 6723 Bertner Avenue, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

LAURENTIAN HORMONE CONFERENCE—PROGRAM FOR 1956

The 1956 Laurentian Hormone Conference of the AAAS will be held at Mont Tremblant Lodge, Mont Tremblant, Quebec, during the period *September 2–7*, and investigators interested in attending this conference should make application to the Committee on Arrangements of The Laurentian Hormone Conference, 222 Maple Avenue, Shrewsbury, Massachusetts, at an early date and in any event no later than *May 7, 1956*. A conference rate of $12.00 per person per day is extended to all invited participants. Attendance is limited to invitations issued by the Committee on Arrangements.

PROGRAM

I. *Neurohumoral-Endocrine Relationships*

Biochemical, physiological and pharmacological aspects of serotonin.
S. Udenfriend, P. A. Shore, C. F. Bogdanski and B. B. Brodie, National Institutes of Health.

Sunday evening, September 2

Endocrine function following transections of upper brain stem and spinal cord.
E. Anderson, R. W. Bates, E. Hawthorne, W. Haymaker, D. McK. Rioch, W. T. Spence, and H. Wilson, National Institutes of Health.

Monday morning, September 3

The influence of the central nervous system on pituitary function.
Monte Greer, Veterans Administration Hospital, Long Beach, California.

Monday morning, September 3

Hormones and rhythms.
Curt P. Richter, Johns Hopkins Hospital, Baltimore, Maryland.

Monday evening, September 3

II. *Hormone Transport in Circulation*

The interaction of thyroid hormones and protein in biological fluids.
J. E. Rall and Jacob Robbins, National Institutes of Health.

Tuesday morning, September 4

The binding of steroids and their conjugates to human plasma proteins.
A. A. Sandberg and W. R. Slaunwhite, Jr., Roswell Park Memorial Institute.
Tuesday morning, September 4

III. *Aspects of Reproduction*

Some experimental studies on the mechanism of mammalian ova implantation.
M. C. Shelesnyak, The Weizmann Institute of Science, Israel.
Tuesday evening, September 4

Progestines in the normal menstrual cycle.
John Rock, Free Hospital for Women, Brookline.
Wednesday morning, September 5

The metabolism of progesterone and its clinical use.
M. E. Davis, University of Chicago Medical School.
Wednesday morning, September 5

Long-acting steroids in reproductive processes.
Karl Junkmann, Schering, A. G., West Berlin, Germany.
Thursday morning, September 6

IV. *Hormone Chemistry and Metabolism*

Glucagon, a second pancreatic hormone.
P. P. Foa, The Chicago Medical School.
Thursday morning, September 6

Insulinase and insulinase inhibitors.
I. Arthur Mirsky, University of Pittsburgh.
Thursday evening, September 6

V. *Hormones and Stress*

Endocrine responses in surgery.
Francis Moore, Peter Bent Brigham Hospital, Boston.
Friday morning, September 7

Adrenal influences upon stomach and gastric responses to stress.
Seymour J. Gray, Peter Bent Brigham Hospital, Boston.
Friday morning, September 7

BIOLOGICAL PHOTOGRAPHIC ASSOCIATION

The 26th Annual Meeting of the Biological Photographic Association, Inc., the first five-day meeting in the group's history, will be held *August 27–31* in the Powers Hotel, *Rochester, N. Y.*

The Rochester Meeting will bring together several hundred medical and other biological photographers and scientists from various sections of the country. A number of important papers will be presented dealing with advances in biological photography. There will be a special award for the best paper presented at the Meeting.

A special day-long workshop will be held Monday, August 27, on recent developments in materials and processes for color photography. Lectures and demonstrations by recognized experts will be tailored to the limitations of the average medical or biological laboratory.

MARTHA M. BRUNINGS, *Secretary*,
343 State Street,
Rochester 4, N. Y.

BOOK REGISTER

Androgens: Biochemistry, Physiology, and Clinical Significance. By RALPH I. DORFMAN, Ph.D., Associate Director of Laboratories, Worcester Foundation for Experimental Biology, Research Professor, Boston University School of Medicine, Boston, Mass., and REGINALD A. SHIPLEY, M.D., Director, Radioisotope Unit, VA Hospital, Associate Professor of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio. This is the first publication devoted specifically to the broad subject of androgens. The available literature on the male hormones and related steroids is compiled, summarized and evaluated The clinical aspects of androgens are well presented, both for the general practitioner and the specialist. Various actions of androgens on sex specific and other tissues are discussed. There is a section on nomenclature; 607 pages; figures and tables; 1956. John Wiley & Sons, Inc., New York, N. Y. Price $13.50.

Diseases of the Endocrine Glands. Second edition. By LOUIS J. SOFFER, M.D., Clinical Professor of Medicine, State University of New York College of Medicine, New York City, Attending Physician, The Mount Sinai Hospital, New York, and ARTHUR R. SOHVAL, M.D., Associate Attending Physician, The Mount Sinai Hospital. This edition of a well regarded book has been revised and brought up to date. It covers all clinical endocrine diseases and their management. Physiologic considerations, diagnosis and treatment are discussed thoroughly. There is a chapter giving complete instructions for making endocrine tests and interpreting the results. Extensive bibliography; 1032 pages; 102 illustrations and 3 plates in color; 28 tables, 1956. Lea & Febiger, Philadelphia, Pa. Price $16.50.

Experimental Tuberculosis: Bacillus and Host—with an Addendum on Leprosy. A Ciba Foundation Symposium, edited by G. E. W. WOLSTENHOLME, MARGARET P. CAMERON and CECILIA M. O'CONNOR. Chairman, A. R. RICH, M.D., Director of the Department of Pathology, The Johns Hopkins University, Baltimore, Md.; and 19 other participants. Basic inquiries into the constitution and properties of the tubercle bacillus which endow it with the capacity to act upon the body as it does, and a consideration of the reactions of the body to those pathogenetic qualities; 408 pages; figures and tables; 1955. Little, Brown and Company, Boston. Price $9.00.

Hebrew Medical Journal. Semi-Annual Publication, Volume 2, 1955, edited by MOSES EINHORN, M.D. Includes articles on mortality in Israel, Maimonides "The Art of Cure," endocrinology in the Bible, and the physician in Jewish law and religious literature. Editorial office—983 Park Avenue, New York 28, N. Y.

Memoirs of the Society for Endocrinology

No. 4. Comparative Physiology of Reproduction. Edited by I. CHESTER JONES and P. ECKSTEIN. This memoir is based on the proceedings of a symposium held in July 1954 at the Department of Zoology of the University of Liverpool. Part I reviews reproductive patterns in fish, amphibia, reptiles and mammals, and includes separate accounts of the evolution of viviparity and vertebrate gonadotropins. Part II deals with the effects of sex hormones in different groups of vertebrates. The volume provides a unique comparative survey of reproductive processes in the vertebrates; 263 pages; numerous illustrations (including a color plate) and tables; 1956. New York: Cambridge University Press. Price $8.50.

No. 5. Hormonal Control of Water and Salt-Electrolyte Metabolism in Vertebrates. Edited by I. CHESTER JONES and P. ECKSTEIN. This memoir is based on the proceedings of a symposium held in July 1954 at the Department of Zoology of the University of Liverpool. It provides a comprehensive survey of water and salt-electrolyte metabolism and its control in different vertebrates. It includes contributions on the problem of the role of the adrenal and other endocrine organs in such control; 135 pages; figures and tables; 1956. New York: Cambridge University Press. Price $4.75.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th St., New York 36, N. Y.

Gestation. Second Conference, March 8–10, 1955, Princeton, N. J. Edited by CLAUDE A. VILLEE, Ph.D., Assistant Professor of Biological Chemistry, Harvard Medical School, Boston, Massachusetts. Contents—Self-regulatory functions during gestation and lactation, social reactions of pregnant and lactating rats, delayed implantation in mammals, morphology and physiology of the uteroplacental circulation,

uteroplacental circulation in mammals, pressures in the fetal circulatory system of the sheep, and indiscriminate distribution of arteries and veins in the mammalian placenta; 262 pages; 118 figures (including a color plate); 1956. Price $5.00.

Shock and Circulatory Homeostasis. Fourth Conference, December 6–8, 1954, Princeton, N. J. Edited by HAROLD D. GREEN, M.D., Professor of Physiology and Pharmacology, and Associate in Internal Medicine, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C. Deals with the action of epinephrine in man, the circulation in the periphery, mesenteric lymphatic dynamics in the rat, the circulation in the splanchnic area, the pulmonary circulation as such and in hemorrhagic shock, and the aortic and coronary blood flow; 291 pages; 69 figures; 26 tables; 1955. Price $5.00.